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PHYSIOLOGICAL REVIEWS

VOL. IV APRIL, 1924 No. 2

ADRENALECTOMY AND THE RELATION OF THE ADRENAL BODIES TO METABOLISM

G. N. STEWART

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In the preface to his monograph on the secretion of urine, Cushny complains that the "increase in bulk of the literature on the kidney has not gone along with an improvement of quality, but rather the reverse. No other organ of the body has suffered so much from poor work as the kidney, and in no other region of physiology does so much base coin pass as legal tender." He has himself nailed not a few of the bad shillings to the counter, and the considered judgment of such an authority must carry great weight. Nevertheless the present writer cannot refrain from respectfully and most regretfully suggesting that perhaps the claims of the suprarenal to share on at least equal terms the bad eminence of the kidney were not fully considered.

Be that as it may, the literature so-called of the physiology and pathology of the adrenal bodies presents a very confused and baffling picture, which only begins to clear somewhat when it is recognized that no mean proportion of the total mass of printed matter, and the portion which contributes most to the haze, can and ought to be stricken from the record on internal evidence alone. Apart from the inherent difficulties of the subject, probably the most important single cause of the state of affairs alluded to is one which may seem rather far-fetched to readers who have not studied the matter for themselves, because it involves a possibility of error so gross and apparently so obvious that it might seem reasonable to assume that the greatest pains would always be taken to avoid it. Unfortunately it is not so. This cause of error is simply the very common neglect of the development of an adequate surgical technique on the part of those who have undertaken to investigate the functions of the adrenals including their relation to metabolism. It is of the utmost importance to recognize that in most

animals the removal of these glands, in such a way that the phenomena which really result from their loss are not overlaid by the direct effects of the operation as such, requires the greatest attention and skill on the part of the operator, and that the observer must be constantly on his guard against assuming without proof that the changes which he sees after adrenalectomy are necessarily due to loss of function of the glands. Nowhere, perhaps, in experimental work is it more necessary to remember the difference between *post hoc* and *propter hoc*. Anyone who looks into the literature will see how often this rule has been neglected, and how often a promising investigation has made shipwreck because the preliminary operation, on which everything depended, was not properly performed. Not infrequently the operation for the elimination of adrenal functions, although perhaps well enough done from the surgical standpoint, has been quite inappropriate from the physiological point of view. For example, in order to eliminate the function of the adrenals, the splanchnic nerves have been cut on both sides. This, of course, involves interference with the innervation of many organs besides the adrenals, including the liver, and does not eliminate the cortex. The common method of eliminating the function of the medulla has been to shear away the adrenals *in toto* and to assume that it did not matter that the cortex was taken away also.

In a review of the relations of the adrenal bodies to metabolism it may seem somewhat reminiscent of snakes in Iceland to begin by stating that we have little definite knowledge of such relations. Nevertheless all that is known of the physiology and pathology of these glands indicates that relations of the greatest importance do exist, and that when they are destroyed the metabolism must be profoundly influenced, if not in its general course then in some one or more of its special features. So that no apology need be offered for giving a somewhat liberal interpretation to the allotted theme. No attempt will be made to cite the literature with any completeness. That is the function of large monographs, such as those of Vincent and Biedl.

The writer hopes that the brevity with which conclusions are necessarily presented owing to limitations of space may not to some readers appear to savor of dogmatism. To forestall such criticism he desires to say without being besought (as the Presbyterian divines were besought by Cromwell), that he freely admits the possibility of his being mistaken.

Consequences of removal or destruction of the adrenals. It is necessary always to distinguish between the cortex and the medulla, or

more generally between the interrenal and the chromaphil tissues. The simplest, which is also the most probable view, is that in the adrenal we have to deal with two glands, distinct in origin, distinct in structure, distinct in function, so far as any function can be attached to them. The anatomical relations are more or less close in the different groups of animals, but there is no evidence that this has any functional significance.

If there are two perfectly distinct glands in the adrenal the function of each ought to be investigated separately. But that is not the way the subject has developed. It is not at all uncommon for physiologists and very common for clinicians even now to speak of "adrenal" function, of an increased activity of the "adrenal," of an increased "adrenal" discharge, when they are referring solely to the medulla. And Biedl has complained justly that pathologists often continue in practice, although not of course in theory, to look upon the adrenal as a single organ.

Addison's observations, published in 1855, *On the constitutional and local effects of disease of the suprarenal capsules*, were the foundation of our knowledge of the consequences of extirpation of the adrenals in animals. For they stimulated experimental investigations which, at first most confused and conflicting in their results, have at length with the improvement in technique tended in the hands of competent observers to a satisfactory degree of uniformity. It is instructive to compare the statements of different investigators as to the results of adrenalectomy on one and the same kind of animal. It is now easy to see that the main discrepancies arose from differences in the quality of the surgery, in the nature and duration of the anesthesia, in the attention given to avoidance of undue loss of heat, in the nutritive condition of the animals, in the way they were taken care of before and after the operation. These circumstances in general affect small animals in a greater degree than large, and the discrepancies are usually more striking in the smaller animals, like rats, guinea pigs and rabbits. A secondary cause of disagreement was that not all workers were equally careful to remove or destroy the whole of the adrenal tissue and to control its complete removal by adequate post-mortem examination. The fact is that the difficulty of performing the operation properly, particularly in such animals as rabbits and guinea pigs, and the amount of training necessary to render the work worth while, have scarcely been realized by some observers who have published statistical results of the consequences of adrenalectomy. Luckily, any

dispute of this kind can always be decided in favor of the worker whose animals live longer or die less frequently, provided he has removed the whole of the adrenal tissue.

With the best technique nearly all dogs die after double adrenalectomy at one sitting in a few days at longest. Strehl and Weiss, (1) whose results were relatively better on dogs and cats than on the smaller animals, as is readily intelligible when the surgical technique is mediocre, give 22 to 138 hours as the limits for their series of dogs operated on at one sitting, and 109 to 124 hours when an interval of months elapsed between the removal of the two glands. In Biedl's (2) hands the results were no better, in those of Langlois (3) worse. So that in the dog the complete operation is very deadly. Exceptionally long periods of survival occasionally reported, as by Pal, are probably due to incomplete removal of the main glands or to the rare presence of accessories, although a perusal of the literature leaves one in doubt.

In cats the most trustworthy results are probably those of Elliott (4). Of 21 animals in which both glands were excised at one operation, 19 died in from 18 to 48 hours (generally on the second day). Strehl and Weiss give 15 to 47 hours as the limits (for 17 animals). It is more important to know the duration of survival which can be expected in the majority of the animals of a series than the minimum and maximum limits, if useful results are to be obtained on the metabolic and other consequences of loss of the adrenals. In Elliott's series of 25 cats from which the glands were removed in two operations, 9 died on the 2nd or 3rd day, 13 lived for 6 to 10 days: 2 survived to the 22nd and 23rd day, and one seemed to have recovered completely when it was killed in the 9th week. Elliott attributes the longer periods of survival to compensatory hypertrophy of small accessory adrenals of cortical tissue. This explanation is also commonly given for the longer survival when the operation is performed in two stages.

Another factor, however, is the shortening of the operation, including the anesthesia, and the diminution of the effects of trauma. This is likely to be especially important in the case of a slow operator working with small animals. For instance, I have had the opportunity to note that the results of a highly skilled operator when removing both glands at one sitting from white rats of a given lot were not only conspicuously better than those of another operator of considerable experience who was performing the adrenalectomy in two sittings on rats of the same lot, but not greatly inferior to his own results when the operation was done in two stages. The feeding before the operation and the after-

care, which are known to be important, as well pointed out by Lewis (5) and by Scott (6), were the same for all the rats. Boinet (7), whose technique for the relatively simple operation of adrenalectomy in rats, must have been sufficiently good, stated nearly 30 years ago that his results on wild (sewer) rats were the same whether the glands were excised simultaneously or in two stages. Of 75 rats one-half survived, and many were in excellent condition 5 or 6 months later. Under good conditions and with good stock 50 to 70 per cent of a large batch of white rats may survive apparently for an indefinite time. In some lots, the percentage may be still greater. Statistics will always vary to some extent according to the number of days or weeks which are taken as indicating complete recovery and capability of indefinite survival. For a certain death rate is unavoidable in any large stock of rats, and it will sometimes be impossible to determine whether a death was due to loss of the adrenals or not.

Of adult rabbits not less than 25 per cent, under the best conditions considerably more, recover completely after double adrenalectomy and live indefinitely. A large additional number survive for some weeks.

The guinea pig has been considered the ideal mammal for testing the indispensability of the adrenals because, according to Biedl, "no author has reported the survival of a guinea pig after removal of both glands," whether in one or in two sittings. Usually the period of survival was only a few hours. Small accessory adrenals may be present, though rarely, e.g., on the vena cava, especially on the right side. But although they may undergo hypertrophy they never seem to be able to grow sufficiently to replace the function of the main glands. The statement of some writers (e.g., Abelous and Langlois) (8) that it is practically impossible to extirpate the right gland completely is quite incorrect, although even the most experienced operators will occasionally be forced to leave the extirpation incomplete on the right side in guinea pigs and rarely in rabbits. Nearly all the results of adrenalectomy on the guinea pig reported in the literature are devoid of value as far as the periods of survival are concerned. These are not related to the loss of the adrenals but to the employment of poor surgical technique in an animal which is at best a bad operative risk.

Much longer periods of survival have been seen in a series of guinea pigs operated on by Rogoff in this laboratory. With an interval of about 2 to 3 weeks between the two operations, out of 17 guinea pigs, 3 lived one day. In 2 of these cases the lungs were congested, and there can be scarcely any doubt that the operation as such was, mainly

at any rate, responsible for the short period of survival. Two of the animals, which happened to be pregnant, lived 2 and 9 days respectively. Three animals survived for 4 days, and three for 5 days. Two of them lived 6 days, one 8 days, one 11 days, one 12 days. One guinea pig survived 28 days. Careful macroscopic search at autopsy failed to discover any accessory tissue. In every case but one the necropsy verified the complete removal of both adrenals. In that animal, which survived 4 days, a small portion (about one-eighth) of the right gland was knowingly left because of the danger of wounding the cava, but it was completely tied off.

In 3 guinea pigs adrenalectomy was performed in one sitting. One of the animals died in 1 day, one survived 5 days, and the third 8 days.

In frogs the complete operation appears to be uniformly fatal, the period of survival being variously stated. Abelous and Langlois (9) found that hibernating frogs might live as long as 12 or 13 days, whereas summer frogs on the average never survived more than 48 hours. But as the glands had to be destroyed by the cautery, it is difficult to be sure that the extirpation was always complete.

The explanation commonly given of the great difference between rabbits and rats and the other animals referred to in the consequences of removal of the adrenals is that small masses of cortical tissue (accessory adrenals) exist in various situations outside of the main glands in a considerable proportion of rabbits and a very large proportion of rats. These by undergoing hyperplasia and hypertrophy are assumed to compensate for the loss of the adrenal cortex, especially when a considerable interval elapses between removal of the two glands. The fact that such hypertrophy does occur not only in the accessory tissue but also in the main adrenal when one has been removed, or in a stump of cortical tissue left by incomplete removal of the adrenals is strongly in favor of this view. For it indicates that the organism does attempt, when it gets the chance, to supply the physiological deficiency occasioned by loss of the adrenals in a most obvious and direct manner by compensatory overgrowth of the accessory tissue when this is present. It cannot, however, be stated that accessory adrenal tissue has been proved to be present in every rabbit or every rat which survives removal of the main glands, or that it is present in sufficient amount to prevent death. Nor can it be stated that it is absent in every cat, dog or guinea pig which dies after complete adrenalectomy. Occasionally, as already mentioned, an accessory has been found in cats, guinea pigs and perhaps in dogs.

It would be unreasonable to stress this point, as it is an impossible task to examine large numbers of animals for microscopic "rests" of cortex. Nevertheless, the assumption that the loss of the adrenals is a far less serious thing for rats than for cats or guinea pigs because of the accessory tissue in the former is still an assumption, however plausible, till it has been shown that the removal of the accessory tissue is incompatible with life. Boinet (10) removed what he could of it in rats without effect, but these experiments are not conclusive. The possibility should not be forgotten that as there are differences in the susceptibility of different animals to removal of the thyroid not connected with the presence or absence of accessory thyroid tissue, the same may be true of the adrenals. The loss of one adrenal never of itself causes death in any animal investigated. Deaths following this operation are due to other causes than adrenal deficiency.

If in the light of the data summarized above we examine the statistics of most of the older observers and also of some recent workers, we cannot fail to be struck with the fact that many of the deaths attributed to loss of the adrenals must have been due to operative shortcomings and other unfavorable extraneous conditions. For example, in one of his early papers Brown-Séquard (11) stated that 44 rabbits, 9 guinea pigs, 3 rats and several dogs and cats all died in 9 to 37 hours after double adrenalectomy at one sitting. For the rabbits and rats and even the guinea pigs, dogs and cats, these results are so extremely bad that we can be certain that practically all the deaths were due to faulty technique and few, if any, to the mere loss of the adrenals. This conclusion is clinched by the fact that when he removed only one gland from 16 rabbits, 5 guinea pigs, 2 cats and 2 dogs, all the animals were dead in 23 to 24 hours. The deduction is unavoidable that when Brown-Séquard announced that the adrenals were indispensable to life, on the ground of such observations, he was merely making a fortunate guess, unconsciously, of course. This is not said by way of criticism but to emphasize the point already alluded to that in this matter experiments which show a longer period of survival are always right in comparison with those which show a shorter period of survival, on the assumption that in both cases the removal of the adrenals has been complete. Later on Brown-Séquard got somewhat better results, but they were never good.

Many years thereafter, in what Biedl describes as "extirpation experiments completely free from objection," it was still possible for Hultgren and Andersson (12) to report that rabbits after removal of

both adrenals at one sitting only lived 5 to 6 days, although they might survive for months if a long interval were left between the extirpation of the two glands. Still later, in fact only 10 years ago, Freund and Marchand (13) stated that after complete extirpation of both adrenals rabbits die without exception in a short time, and usually on the first day. In spite of all the work which had been done on the rat, going back to Harley's demonstration that survival could occur after double adrenalectomy, ignoring Boinet's conclusion that survival was quite common and that it mattered little for this whether the operation was done in one or in two stages, Schwarz in 1910 announced that rats do not survive more than a day if both adrenals are removed at one time, and in this he was *confirmed* by Kahn and Starkenstein (14). According to them if a shorter interval than from 3 to 4 weeks is left between the removal of the first and second adrenal, death ensues within 2 days after the second operation. In 1911 Biedl reproduced a table giving the results of what he calls "the exact extirpation experiments" of Strehl and Weiss (1). In that table it is stated that after removal of both adrenals death ensued in 26 rabbits in 8 to 14 hours; in 20 guinea pigs in 4 to 9 hours; in rats 15 to 19 hours; mice 8 to 13 hours; frogs 22 to 45 hours. Out of 15 cats not one lived longer than 28 hours. These results are even worse than those of Brown-Sequard obtained nearly 40 years before. Is it not clear that they in no sense represent the effects of adrenal insufficiency, but simply supply data on the ease with which animals, especially small animals, can be killed by bad surgery, with its concomitants and consequences, excessive anesthesia, hemorrhage, trauma, cooling and the shock to which all these factors contribute? It is the uncritical acceptance, citation, reiteration of such results which has so cluttered up the pages of adrenal physiology that the literature has become a nightmare to the serious student. In a quite recent paper (15) it is reported that extirpation of the two adrenals in 8 rabbits at one or two sittings caused death in 15 to 60 hours (average 26 hours). These animals were prepared in order to test the question whether their resistance to morphine was diminished by decapsulation. But how can any useful results on such a question be obtained with a surgical technique which killed all the animals without giving them a chance to develop the consequences of loss of the glands?

Only 9 years ago it was thought worth while by Marie and Morax (16) to report that in their hands bilateral capsulectomy at one sitting in guinea pigs had always been followed by death at the end of some

hours, even in animals in which a fragment of one gland had been spared. All that this proves is that the animals were killed by poor surgery. For guinea pigs with a small portion of one adrenal survive indefinitely; and complete double adrenalectomy at one sitting, when properly done, does not kill the animals in some hours. Many years previously Abelous and Langlois (8) found that adult guinea pigs never lived more than 9 hours (average 5 hours) when both capsules were completely destroyed on the same day. Even when an interval of 8 to 15 days elapsed between the two operations the period of survival was only slightly longer (average 12 hours). This was the case in spite of the fact that these observers did not cut the adrenals out by a clean dissection but crushed or burned them with the actual cautery. They are not ashamed to state that when they employed anesthesia they lost so many of the animals, even after ablation of one adrenal, that for a long time they had renounced the use of anesthetics. One animal in which the second capsule was operated on 13 days after the first, lived 5 days. But *at the autopsy it was found that two-thirds of each gland were intact.* It is surely clear that none of these results, to obtain which 100 animals were sacrificed, throw any light upon the consequences of adrenal deficiency in the guinea pig. The statement made by several writers, of whom Gratiolet was the first, that removal of one adrenal (especially the right) is uniformly fatal in guinea pigs is the exact opposite of the truth. With reasonably good surgery extirpation of one gland is usually as innocuous in the guinea pig as it is now admitted to be in all the other laboratory animals.

It is probably not always realized, especially by young writers, how the rest of their work is prejudiced, sometimes unjustly, by the publication of surgical data, the worthlessness of which can be immediately recognized. When animals die in a day or so after adrenalectomy the loss of the adrenal function, if it contributes at all to the fatal result, is of far less importance than the general effects of the operation. It is surprising how lightly this factor has been esteemed by many observers, who both in acute and survival experiments have confidently attributed everything which happened after the moment at which they ligated, crushed, burned or hacked out the glands, to the loss of their function and to nothing else. Even at the time of Brown-Séquard's experiments, nearly 70 years ago, it was understood and often pointed out that the effects of trauma in removing those organs, lying everywhere in close relation to veritable thickets of sympathetic nerve fibers and ganglia, must always be taken into account.

As to the symptoms produced by total adrenalectomy it is important to note that even in animals like guinea pigs and cats, which are practically all destined to die, there are no symptoms, or none that are at all obtrusive, from the time when the direct effects of the final operation have worn off, until a relatively short time before death. The appetite may be good, the body temperature normal, the muscular activity and strength not sensibly different from that of a control animal subjected to a similar dummy operation. This is also true of the rabbits which eventually succumb; nobody could distinguish them from those which are going to survive indefinitely. There may be some loss of weight, but at this stage this is as likely in the one group as in the other. Then comes a change, often so abrupt that one day the animal may appear to be well and the next day may be obviously sinking, and on that day or the following one it may be dead. The first sign is the refusal of food. The rectal temperature will now be found subnormal. Muscular weakness becomes more and more pronounced. Diarrhea is often present. The animal grows rapidly worse. Presently it is unable to stand, the posterior extremities showing pronounced weakness sooner than the anterior. The respiration fails, often while the heart is still beating fairly well, although the blood pressure is diminished. Not infrequently in rabbits some of these changes may occur temporarily, especially a decided fall in rectal temperature and loss of appetite for a day or two. Then recovery may take place, perhaps succeeded by another fall of temperature and some of the concomitant symptoms, and the animal may eventually recover completely and survive indefinitely. But this is only true when it has not gone far on the downward road. If our interpretation of the phenomena is correct, these symptoms indicate vicissitudes in a process of adjustment which succeeds in the case of some rabbits in compensating for the loss of the adrenals but fails in others. Marine (17) has brought forward evidence that it may be possible to realize in some rabbits a syndrome associated with experimentally produced deficiency of the adrenal cortex, since after removal of the main glands a sufficient amount of accessory cortical tissue may be present in some of the animals to prevent death and yet not enough to completely compensate for the loss of the adrenals. In animals like the cat and guinea pig it has not proved feasible to produce such a condition by removing with the knife a large proportion of the total mass of the glands. The animal either dies as it would have done if total adrenalectomy had been performed, although possibly not so soon, or it recovers completely.

The abruptness of onset of the symptoms which announce a fatal issue is naturally best seen in animals which have survived the final adrenal operation at least for some days and have recovered completely from its direct effects. It differentiates death from adrenal deficiency sharply from death due to loss of the internal secretion of the pancreas, where the animal goes gradually from bad to worse from the beginning to the end, although the characteristic change in the metabolism and its most obvious consequence, the rise in the blood sugar content, are evident in a few, or at least before many hours. The suddenness of onset of the serious symptoms after adrenalectomy is differentiated also from what happens in the case of removal of the parathyroids because here, in the most susceptible kinds of animals, the period of latency is short and does not vary within very wide limits. The peculiarity, in the case of the adrenals—and it has not been sufficiently recognized—is that the animal may be going along well, perhaps weeks after removal of the glands, and then suddenly something happens and it rapidly develops the symptoms which prognosticate death. These symptoms if seen in an animal whose history is unknown may not be sufficiently characteristic to permit a diagnosis of adrenal insufficiency. But when seen in an animal deprived of its adrenals they rarely leave one in doubt as to the cause of death.

What is this something which has suddenly happened? The answer to that question would reveal the secret of the adrenal function. It cannot be given at present. But the problem can be narrowed somewhat. Complete suppression of the output of epinephrin from the adrenals has no noticeable influence on life or health. It may be concluded that the loss of epinephrin from the adrenal medulla has nothing to do with the fatal symptoms of adrenal deficiency. Such statements as those of Vassale and Zanfrognini (18), that in cats and rabbits total extirpation of the medulla causes death in the same way as total extirpation of the adrenals, while after partial extirpation of the medulla the animals lived about 3 weeks and then died of cachexia, must clearly be written off. The fact that the rabbits died as well as the cats indicates that bad surgery and not deficiency of the medulla was the cause of death. The same judgment can be passed even more confidently upon the conclusion of H. and A. Cristiani (19) that rats never survive after partial adrenalectomy unless a portion of medulla in good condition has been left, and that "it is evident that it is to the medullary substance that the essential function of the capsules appertains." For they find that after complete removal of both adrenals

all rats die in 10 to 20 hours after the operation, and it makes little difference whether the operation is done in one or in two stages. There is also no evidence that the extracapsular chromaphil tissue exerts an important or indeed any function. It is not even certain that it gives off epinephrin. Finally there is absolutely no evidence that the function of the extracapsular chromaphil tissue if it has one, breaks down rather suddenly, weeks, it may be, after removal of the adrenals and that this precipitates the fatal result. Nor is death prevented or notably postponed by the administration of adrenalin as continuously as possible. If the animal may seem to rally for a little this is only what is seen in other conditions under the influence of this powerful cardio-vascular stimulant, in doses much larger than there is any reason to believe can be liberated from the chromaphil tissue. The chromaphil system may therefore be excluded from the discussion.

The cortex is the portion of the adrenal essential to life. How it exercises its function is utterly unknown. Two possibilities present themselves. It may form and discharge some substance essential to the maintenance of the body in health, or it may neutralize or aid in the elimination of toxic substances normally produced in the tissues. These two possible functions are not mutually exclusive. An active substance formed in the cortex might be carried to the tissues or to particular tissues and there exert its beneficial action by directly favoring their functional activity, or by helping to neutralize harmful products of this activity, or in both ways. On the other hand, toxic substances might be carried from the tissues to the cortex and there rendered harmless or useful. Both theories have been held and neither can be proved or disproved at present. It must be remembered, however, that when nothing was known of the function of the thyroid or of the internal secretion of the pancreas a favorite assumption was that the serious consequences of their removal were due to the loss of a "detoxicating" action, in the absence of which toxic substances, which could no longer be neutralized, accumulated in the body. As soon as it was established that thyroid "substitution therapy" was a brilliant success, especially when the specific hormone of the thyroid was isolated, nobody talked any more of the detoxicating function of the gland. It is the same now with the pancreas. It was the same for a time with the adrenals after the discovery of epinephrin. When it was seen, after the first enthusiasm had passed, that the discovery of epinephrin had not in any important degree solved the riddle of adrenal function, the ghost of the "detoxication" theory began to

walk again. Anybody who observes closely the symptoms presented by animals dying of deficiency of interrenal (cortical) tissue, must be struck with the fact that they do suggest a profound intoxication. Abelous and Langlois (9), (20) thought that this was due to toxic substances produced especially in the muscles. They asserted that blood from adrenalectomized frogs, guinea pigs and dogs and also alcoholic extracts of their muscles, when injected into normal animals of the same kind, caused symptoms which they interpreted as those of muscular fatigue, and when injected into adrenalectomized animals hastened the onset of death. These conclusions were supported by other observers and the curara-like action of the supposed toxic substances was emphasized. It must again be pointed out, however, that observers in whose hands all guinea pigs died in a few hours after double adrenalectomy and who were unable to remove one adrenal from a guinea pig under anesthesia because of the great mortality were not in a position to detect a shortening of the period of survival due to such toxic substances. Kellaway (21) has recently reinvestigated the alleged muscarin-like action (Loewi and Gottwert) on the frog's heart of serum from frogs moribund or dead as a result of extirpation of the adrenals, and has been unable to confirm its existence. We have gained the impression that it is the central nervous system which is most heavily involved in the intoxication, if intoxication it should be called.

At this point we may revert for a moment to the question why an animal which has apparently recovered after adrenalectomy should rather abruptly develop the fatal symptoms. Is there a store of the cortical hormone, if the interrenal tissue produces such a hormone, which has become exhausted? This seems quite improbable because of the considerable time which may elapse during which the animal is going on well, unless indeed we assume that all animals, including guinea pigs, possess sufficient accessory cortical tissue to keep them going for a time, and that this tissue either uses up its preformed stock of hormone or becomes "exhausted" under the "strain" of providing an abnormally large amount, till the breaking point is reached and the animal begins abruptly to go down hill. Exhaustion under the strain of increased functional work must not, however, be lightly assumed, especially in the case of a tissue with so great a power of compensatory hypertrophy as the interrenal tissue. Another possible assumption, on the hormone theory, is that the nervous system or whatever other tissues are especially affected in the long run by the loss of the adrenals, can go on functioning normally, or at least well. for some time and then break down rather suddenly.

On the intoxication theory it may be supposed, as on the other theory, that if some cortical tissue is always present this suffices for a time to prevent the accumulation of the toxic substances to the danger point, which is quickly reached when the compensation breaks down. Or the nervous or other tissues may hold out for a while, even in the presence of a certain amount of the supposed toxic substances, till the critical concentration is attained at which the symptoms of profound intoxication appear. A third possibility appears to be present on the intoxication theory, that the toxic substances may be eliminated or destroyed by other tissues than the interrenal tissue, e.g., by the kidney or liver, and that after adrenalectomy these suffice for a time to get rid of them completely or to prevent them from attaining a dangerous concentration. The breaking point may be reached either because the kidney, let us say, cannot cope completely with the elimination of the poisons, or because it is itself injured in attempting to do so, or because in some other way the loss of the adrenals affects it unfavorably. The observations of Marshall and Davis (22), who report a retention of urea nitrogen in the blood in cats after adrenalectomy, are suggestive in this connection. The only drawback is the necessarily short period of survival in cats. One-third of their animals lived 5 to 7 days; a satisfactory result, but half of them died in 1 to 2 days after total adrenalectomy in two stages, although their condition is stated to have been excellent after the second operation. Like Marine (17), we have sometimes observed changes in the kidney after adrenalectomy, but do not know whether these might not have been associated in some degree with injury to the blood supply or nerves at the time of the operation, or in the healing process.

If such an organ as the kidney breaks down rather suddenly, the abrupt onset of the symptoms would receive a satisfactory explanation. This theory is perhaps superior to the "hormone" theory, inasmuch as it is known that several of the organs can and do take part in excreting or destroying toxic substances, whereas it is highly improbable that anything deserving the name of a cortical hormone would be formed anywhere except in the interrenal tissue. It would complicate the discussion too much to consider whether this applies even to the interstitial cells of the gonads, but we believe it does. However nearly allied they may be in structure and origin to the interrenal tissue, their functions are not the same and very different effects are caused by their removal.

There is no good evidence that the blood pressure is affected by cortical deficiency until the animal is moribund. Still less is there any proof that after suppression of the epinephrin output alone the blood pressure is at all affected. Nothing closely resembling the classical picture of Addison's disease in man has been observed in animals suffering from adrenal deficiency, nor perhaps could this be expected even if it were clearly ascertained, which it is not, that in Addison's disease the only lesion is in the adrenals and more precisely in the adrenal cortex. For the chronic development of the pathological change is difficult to imitate in animals, although attempts have been made, as by the action of radium emanation and Roentgen rays, as also by experimental infection of the glands. It must not be forgotten that after all the symptomatology of a particular physiological deficiency must often be expected to differ markedly in organisms so different in physiological status and organization as human beings and the ordinary laboratory animals. So that there is no more reason for disappointment at the failure of attempts to reproduce an experimental Addison's disease by creating an adrenal deficiency than at the failure of attempts to reproduce myxedema in similar animals by creating a deficiency of thyroid tissue. As already indicated the likelihood of success in such attempts might be expected to be less in the case of the adrenal cortex than of the thyroid because of the more acute condition which follows the removal of the whole or the greater portion of the gland. That something more than loss of function of the suprarenal bodies or of the cortex is concerned in Addison's disease is indicated by several considerations, which cannot be discussed here. But it may be remarked that among the 11 cases reported by Addison in his original paper [4] showed a lesion affecting only one suprarenal, whereas removal of one adrenal in animals causes no significant symptoms.

If the cortex is the important part of the adrenal it would seem logical in considering the special relations of the gland to metabolism to take the cortex first. It happens, however, that by far the greatest amount of work has been done upon the medulla, the reason being that it forms and gives off a well-characterized substance, epinephrin, which in sufficient doses causes many striking reactions, including definite metabolic effects. It will be convenient therefore to consider first some of the supposed relations of the medulla to metabolism. An important question in this connection is the rate at which epinephrin is ordinarily given off from the adrenals. For if enough of it is given

off, effects upon metabolism may be assumed as a matter of course. Measured under certain experimental conditions (by the rabbit intestine segment method), the average output per kilogram of bodyweight per minute in 103 cats was found to be 0.000226 ± 0.000007 mgm., and for 32 dogs 0.000227 ± 0.0000096 mgm. (23). In nearly 85 per cent of the cases the output lay between 0.00010 mgm. and 0.00030 mgm. The larger and more variable outputs obtained by Kodama (2½ times greater for cats, 4 times greater for dogs) are quite unreliable, as it is only too evident from his paper that he had not adequately mastered the technique of the assay. Differences in the anesthetic and in the operation for collecting the adrenal vein blood had no great effect upon the outputs measured. From our results we concluded that the concentration of epinephrin in the arterial blood could not have been greater at most than 1:500,000,000 to 1:1,000,000,000. Trendelenburg (24), working with another method (frog perfusion) which, however, has the drawback that the serum pressor action is difficult to eliminate completely, convinced himself that if any epinephrin was present in arterial blood the concentration did not at most exceed 1:1,000,000,000. No epinephrin has ever been detected with certainty in the peripheral blood of man or animals in health or disease. In the arteries it is too dilute. Before the blood reaches the veins it has probably been destroyed. Some of the statements in the literature on the concentrations of epinephrin which may exist in the peripheral blood can only be characterized as preposterous. Thus A. Fraenkel (25), by a method which Biedl says is apparently free from objection (rabbit's uterus), obtained numbers from which Biedl calculates that "in the total blood in Basedow's disease 50 to 100 mgm. of adrenalin may be present against 12 mgm. in healthy persons." Now 50 to 100 mgm. of adrenalin in 5 kgm. of blood would correspond to 1:100,000 to 1:50,000 epinephrin. How can anybody who knows the actions of adrenalin suppose for a moment that blood with such a concentration could circulate continually in the vessels of a patient suffering from Graves' disease and that the general blood of healthy persons could continually contain 1:400,000 of epinephrin? In another place, however, Biedl states that he considers "1:400,000 too high since an adrenalin solution of this concentration causes a marked rise of blood pressure, whereas 1 cc. of normal serum is without action." As already stated, this concentration is not only too high but probably at any rate 1000 to 2000 times too high. Many other illustrations might be given of the worthlessness of a great part of the so-called

estimates of the epinephrin concentration and rate of output in the literature. Sometimes such rates have been given as would entail the formation and discharge of the whole store of epinephrin in the adrenals in 10 to 40 minutes. We can only say that there is no evidence that such tumultuous discharges of epinephrin ever occur, or could occur without entailing grave consequences.

The adrenal medulla and experimental hyperglycemia and glycosuria. The view has been expressed by numerous writers that most, perhaps all, of the best known forms of experimental hyperglycemia (and glycosuria) are dependent primarily upon excitation of the adrenal glands to increased secretion of epinephrin, which then causes an accelerated mobilization of sugar in the same way as adrenalin does when artificially introduced. This idea was originated by Blum, following his discovery that the administration of adrenalin causes glycosuria. He suggested that the piqûre glycosuria described by Claude Bernard in 1855 might really be an adrenalin glycosuria due to stimulation through the nervous system of the output of epinephrin from the adrenals. And so it might and must be, in part at least, if the output is stimulated to a sufficient extent to raise and keep raised the concentration of epinephrin in the blood to the level attained when glycosuria is produced by the subcutaneous injection of adrenalin or its continuous infusion into a vein. All that was lacking was proof that such an outpouring did occur, and this was never furnished then or since. The statement of Waterman and Smit (26) that after piqûre epinephrin can be detected in the general venous blood is simply based upon an erroneous interpretation of the so-called Meltzer-Ehrmann (frog's eyeball) reaction, which is useless for such a purpose. Concentrations of epinephrin in venous blood which could be detected by so insensitive a test object would cause enormous, probably fatal, physiological reactions, and would imply an incredible rate of output. Even in the arterial blood and with a much more sensitive method (frog perfusion) no epinephrin has been found. Several observers have more recently reported results indicating an increased output. But in some cases this conclusion is based simply upon lack of attention to established facts. Thus Houssay and Cervera (27) performed piqûre, then clipped the adrenal veins, and on releasing them obtained constriction of the denervated limb. They conclude that "these experiments demonstrate that piqûre causes an intense and prolonged discharge of adrenalin." But they forget that when the veins are clipped and then released without piqûre the spontaneous liberation of epinephrin causes a similar

effect, and they mention no comparative observations. The best paper is that of Carrasco-Formiguera (28) from Cannon's laboratory. He employed the denervated heart reaction in a way which, as pointed out by us, is capable of yielding unequivocal, although probably not very exact quantitative results (collection of adrenal vein blood in a cava pocket for a definite time and subsequent release, *so that the only variable affecting the heart is the adrenal blood*). He found that piqûre causes a temporary increase in epinephrin output but made no quantitative measurements. He points out clearly that his result would by no means prove that piqûre hyperglycemia is due to this, even if it had not been shown that the hyperglycemia is well obtained in the absence of adrenal epinephrin. Rogoff has found that acute cerebral anemia causes increased epinephrin output. Whether piqûre acts in the same way by rupturing blood vessels and thus causing anemia of neighboring structures, or by rupturing or stimulating nerve paths mechanically is undetermined. As it affects the activity of some splanchnic fibers it might be thought probable that the epinephrin-secretory efferent path would be affected also. But only actual experiments avail to settle such matters.

It must always be remembered that it would not be enough to demonstrate an increased output following piqûre, it must be shown that the output is so greatly increased and for so long a time as would be necessary to cause an epinephrin hyperglycemia of the magnitude and duration observed.

The question has been settled by the demonstration that when the adrenal epinephrin output has been suppressed by removal of one adrenal and section of the nerves of the other in cats, or by removal of both adrenals in rabbits, hyperglycemia is caused by piqûre just as in control normal animals and is not noticeably different in magnitude or duration (29). The conditions which must be observed if such experiments are to be properly performed were pointed out and the negative results of some previous workers were traced to lack of observance of one or more of these conditions. Thus Mayer (30) worked with rabbits immediately after extirpation of the adrenals. No time for recovery was allowed as the observations only lasted 8 hours. He examined only the urine for sugar (qualitatively). In Jarisch's (31) rabbits the glycogen store was uniformly deficient, and so on. Recently Houssay (32) has confirmed our result (on 2 adrenalectomized rabbits).

The hyperglycemia caused by ether and by asphyxia, attributed by numerous writers to increased output of epinephrin, has been shown similarly to have no essential relation to the adrenal or in particular to the adrenal medulla, and is not an epinephrin hyperglycemia (33). In the case of ether hyperglycemia this conclusion has been confirmed by Tatum and Atkinson (34). It could really be deduced from the fact that under ether anesthesia the epinephrin output is only on the average 0.0002 mgm. per kgm. per minute, which is 20 times less than the rate at which Underhill (35) infused adrenalin continuously into a non-narcotized rabbit's vein without obtaining glycosuria, although he found in confirmation of Ritzmann (36), that these large doses caused some glycosuria in urethanized rabbits, possibly because the urethane itself has already raised the blood sugar content and the adrenalin then readily pushes it above the threshold at which sugar passes into the urine (37). Certainly it is not any stimulation of the epinephrin output to which this effect can be attributed, since the output in urethanized cats was found to be only slightly in excess of that under ether (0.00025 mgm. instead of 0.00021 mgm. per kgm. per minute) (23).

If a true emotional hyperglycemia exists, a question on which there is some difference of opinion (38), it can have little interest in this place. For there is no evidence that emotional excitement affects the metabolism of an animal whose adrenal medulla has been eliminated in any other way than it does a normal animal. No significant difference has been found in the blood sugar content of normal animals (mainly rabbits and cats) collected first when they were quiet and again after they had been "excited" for a time (39). The average blood sugar content was the same in a series of cats whose epinephrin output had been interfered with by excision of one adrenal and denervation of the other (0.095 per cent for 19 observations) as in normal control cats (0.096 per cent for 19 observations, by the Lewis-Benedict method). In another series the average blood sugar content in 5 "quiet" normal cats was 0.082 per cent, and the average in 9 "excited" normal cats 0.077 per cent by the Folin-Wu method. In 25 cats with a complete adrenal operation for interfering with the epinephrin output the behavior of the animal before and during withdrawal of a blood specimen was noted. For 10 "quiet" animals the average blood sugar was 0.065 per cent, and for 15 "excited" cats 0.070 (Folin-Wu method). For 22 cats with a complete adrenal operation as described the average blood sugar was 0.073 per cent, and for 16 normal controls 0.079 per

cent (Folin-Wu method). The average for 12 adrenalectomized rabbits was 0.106 per cent, for 6 control rabbits 0.106 per cent (Folin-Wu method), precisely the same. The average for 7 dogs subjected to a complete adrenal operation was 0.083 per cent, and for 6 control dogs 0.082 per cent.

It is really time for writers to stop quoting, at least in connection with the adrenal medulla, the conclusion of Porges (40), previously announced by Bierry and Malloizel (41), that a marked hypoglycemia exists in dogs after adrenalectomy. The animals only lived a short time and a hypoglycemia, even if present, would have little significance.

Other forms of experimental glycosuria which have been supposed to depend upon the adrenals are those produced by diuretin (Nishi) (42) and by caffein (Pollak) (43). Until they have been reinvestigated with precautions to avoid errors already dealt with and with estimation of the blood as well as the urinary sugar, judgment must be suspended. According to McGuigan, salt glycosuria can be easily obtained in adrenalectomized cats and dogs and phlorizin glycosuria in adrenalectomized rabbits (44). One form of drug hyperglycemia, that produced by morphine, is especially interesting because it seems to be connected in some way with the adrenals (39), and perhaps especially with the medulla. The suggestion of Houssay and Lewis (46) that in operating on the adrenals we injured the splanchnic nerves unwittingly cannot be accepted for reasons which seem sufficiently obvious. One is that the operations were the same as those in the experiments on piqûre hyperglycemia, which showed that the increases in the blood sugar were of the same order as in the control animals.

One other remark may be made, or rather a question asked. Some writers claim that the extracapsular chromaphil tissue is not only of functional importance but is as important as the adrenal medulla, or more so, and can act vicariously for the medulla in its absence. At the same time these writers, or some of them, agree that increased output of epinephrin is the cause of many of the experimental hyperglycemias and of such reactions as acceleration of the denervated heart, or vasoconstriction of a denervated limb following stimulation of afferent nerves, because these reactions are alleged to be abolished by exclusion of the adrenal medulla, while the extracapsular chromaphil tissue by hypothesis continues to function. How do these intellectual acrobats manage to ride both horses at the same time?

The adrenals and glycogen formation. It has often been asserted that the power of the liver to form and store glycogen is impaired by interference with the adrenals. The loss of the epinephrin output has been generally considered responsible. It may be stated with confidence that the negative results reported (such as those of Schwarz and of Kahn and Starkenstein) (14) were all due to failure to observe essential conditions. Porges' (40) dogs, which the student of the relation of the adrenals to carbohydrate metabolism has on his back all the time like black care, were practically dying animals; they had undergone a severe operation, were not eating, and it is not necessary to drag in the loss of epinephrin to account for a reduced glycogen store. It has been demonstrated that the glycogenic function of the liver is not essentially modified by complete adrenalectomy in rabbits and rats nor by elimination of the medulla in cats (29). I do not enter into the question whether there may not be a period after removal of the adrenals in rats and perhaps in rabbits where a condition of deficiency of cortical tissue may, as such, affect the glycogenic function because I do not see how to disentangle such a factor from the general condition of the animal. The problem is precisely the same when the so-called resistance of animals to poisons is tested after adrenalectomy.

The adrenal medulla and pancreatic diabetes. The theory that epinephrin is a physiological antagonist of the pancreatic hormone, and that when the pancreas is removed diabetes follows owing to the unchecked action of epinephrin given off from the adrenals (Zuelzer (47); Eppinger, Falta and Rudinger (48)) has so flimsy a foundation that it is surprising that it should have had for a time so wide a vogue. Even as regards the mere symptom hyperglycemia, all that the theory had really behind it was the fact that in large doses, especially when introduced under the skin, adrenalin does cause a hyperglycemia. Its sponsors never proved, nor did they apparently think it obligatory to prove, that the necessary amount of epinephrin was or could be given off from the adrenals. It has already been pointed out that elimination of the adrenal medulla causes no demonstrable effect upon the blood sugar content. This is of itself sufficient to strike the props from under the theory of Zuelzer. His statement that a pancreatic extract is capable of suppressing adrenalin "diabetes" is no proof of a specific physiological antagonism, and it has been shown by Macleod and his collaborators (49) that insulin not only prevents adrenalin hyperglycemia but also the hyperglycemia caused by mechanical asphyxia, carbon monoxide, ether and piqûre. We have found that the same is true of morphine hyperglycemia (45).

Attempts to support the theory by showing that removal of the pancreas does not cause diabetes in the absence of the adrenals reveal an extraordinary lack of appreciation of two fundamental facts: first, that animals dying after total adrenalectomy, superadded to total pancreatectomy, are singularly unfavorable subjects in which to study the presence or absence of pancreatic glycosuria or hyperglycemia; and second, that diabetes is much more than hyperglycemia and glycosuria, it is a disease which cannot be produced by epinephrin but is always produced by removal of the pancreas. Nothing can therefore be deduced from the fact that a dog with the adrenal veins tied off did not develop glycosuria in the 36 hours for which it lived (47), or from the record of surgical failures, in dogs and cats, which constitute Mayer's contribution to the subject (50). Hédon and Giraud (51) and Hédon (52) have probably made the best observations on dogs, in which total pancreatectomy was combined with total adrenalectomy in stages. But these operations do not permit survival of the animals for a sufficient time to yield results which have any special significance. Certainly the observations do not justify the conclusion that a functional relation exists between the pancreas and the adrenals.

When the medulla alone is eliminated by operations already mentioned and the animals (dogs) allowed to recover completely, subsequent ablation of the pancreas is followed by all the symptoms of pancreatic diabetes, including marked hyperglycemia and glycosuria of the same order as in the controls (53). The blood sugar content and the clinical condition are affected by insulin just as in the control diabetic dogs. The animals may survive for weeks. Even after removal of the (already demedullated and denervated) remaining adrenal, the diabetes persists in undiminished intensity, so long as the animals remain in good condition. Houssay and Lewis (54) also showed (in 2 dogs) that after extirpation of the medulla pancreatectomy caused diabetes during the time the animals continued to live (8 and 3 days).

It can be concluded then that the active substance of the adrenal medulla is not essentially concerned in the production of pancreatic diabetes. This probably applies to the cortex also, but the proof is much less complete in this case because of the short period of survival of the animals after complete adrenalectomy. On the other hand, the pancreatic hormone (insulin) does not appear to have any close relations to the adrenal medulla. The theory that pancreatic diabetes is an adrenalin diabetes was never supported by any real proof, and if still held by anybody should be abandoned. For a trenchant criticism of

such so-called pluriglandular theories of diabetes, which may well serve as their funeral sermon, a recent paper of Allen (55) is to be recommended. Lusk and Riche (56) have shown that not one of the statements as to the supposed rôle of epinephrin in metabolism and its action upon other endocrine organs (pancreas and thyroid), upon which the crude schemata of Eppinger, Falta and Rudinger were based, is true; that there is no evidence that the rate at which glycogen is transformed into dextrose or the rate at which dextrose is oxidized in the organism is influenced by epinephrin liberated from the adrenals; and that their theory is not tenable in any of its particulars.

The action of insulin on the blood sugar in adrenalectomized rabbits does not differ noticeably from its action in normal rabbits (57). This might be expected since adrenalin hyperglycemia, requiring for its production much larger quantities of adrenalin than are given off from the adrenals, is easily counteracted by insulin. Even if the small physiological output of epinephrin did slightly delay the insulin effect, or slightly alter the rate of decline in the blood sugar or the minimum attained, such an influence would be difficult to detect because of the variability of the curve in different animals. A recent statement (58) that there is a detectable difference in adrenalectomized rabbits is discounted by the maladroit manner in which the animals were prepared as well as by the absence of proper data on the blood sugar. The statement that the "mortality was truly great" in rabbits decapsulated at two sittings, unless the cortex was simultaneously transplanted, when all the animals lived, is illuminative in regard to the surgical technique. In the only experiment described in detail the insulin observations were began on the *third day* after the last operation. This is not permissible.

Cannon, McIver and Bliss (59) have put forward the hypothesis, which may be considered an elaboration of the theory of Zuelzer, that when the blood sugar tends to fall the output of epinephrin is stimulated, constituting a delicate regulatory mechanism which safeguards the constancy of the blood sugar content. Their evidence for the increase in epinephrin output is that when the blood sugar content falls under the influence of insulin in normal cats the rate of the "denervated" heart increases. When glucose is now injected the acceleration in the heart rate disappears. While it makes poor music to harp upon one string, it seems necessary to point out again that it is very risky to assume that changes in the rate of the denervated heart occurring under such complex conditions, in the midst of such metabolic disturbances, are necessarily due to alterations in the rate of epinephrin output. A complete

check is not afforded by the reported observation that when one adrenal gland is removed and the other denervated or when both glands are removed the blood sugar is diminished under insulin but the heart rate does not increase. If this is so, it would be further evidence that the extracapsular chromaphil tissue is not of the same physiological value as the adrenal medulla. The total adrenalectomy, it is to be supposed, was performed shortly before the insulin was administered, and we have already shown that certain changes in the rate of the denervated heart attributed by Cannon to increased epinephrin output may sometimes fail to be elicited immediately after adrenalectomy in an acute experiment, and may yet be well obtained after a short interval.

Ott and Scott (60) asserted that glucose increases the amount of epinephrin in the blood, "as shown by the intestinal strip of the rabbit." "Hence," they proceed, "we have here a circle: epinephrin stimulates the secretory nerves of the adrenals to produce epinephrin, which *via* the glycogen of the liver produces more glucose, which in its turn generates more epinephrin." The other sugars also increase the amount of epinephrin in the blood. The reader may wonder why the blood does not turn into syrup until he realizes that this is simply a picturesque flight of the imagination. The method, as employed, could yield no information.

On Cannon's theory, it would seem that the output of epinephrin measured by us (23) must be too small and not too large, as Cannon has previously maintained, since we employed anesthetics which cause hyperglycemia. However, it is unnecessary to carry the discussion farther upon this line. The best evidence that the epinephrin output exerts no important or indispensable function is that after its suppression the animals do not differ notably from normal animals in their blood sugar content, in their power to form glycogen, their power to mobilize sugar in response to piqûre, etc., their resistance to fatigue, in short, in their capacity to meet all the emergencies of life.

As regards possible relations of the medulla to the general metabolism, we have nothing but speculations. It has been supposed to be linked in a special manner to the thyroid, which of course exercises a highly important metabolic function. Up to the present, however, the linkage has been established by drawing triangles or polygons on paper to indicate how these two glands and the pancreas and other endocrine tissues may be conceived as acting upon each other rather than by demonstrating that any such functional connection actually exists in the body. The statement of Asher and Flack (61), confirmed by Levy (62), that

the pressor action of adrenalin is enhanced by thyroid activity, has no bearing upon the question unless it can be shown that this is true of the epinephrin actually given off from the glands. Now most observers (63) agree that suppression of the epinephrin output leaves the blood pressure unaffected, and it seems idle to assume that a pressor action which cannot be demonstrated would be still feebler were it not increased by a supposed action of the thyroid. According to Lieb and Hyman (64), the sensitization to adrenalin seen by Asher and Flack and by Levy has been misinterpreted by them and is not due to stimulation of the thyroid. Marine (17) was unable to obtain evidence that the heat production (basal metabolism) in rabbits was influenced by interference with the medulla. The supposed relation of the adrenal medulla and the thyroid in regulating the body temperature lacks proof. The rectal temperature is unaffected by operations which suppress the epinephrin output. Morphine, which causes a marked hyperthermia in normal cats, produces quite as great an effect in cats whose epinephrin output has been interfered with.

No space can be given to a consideration of the variations in the epinephrin store of the adrenals studied by Batelli (65), Elliott (66) and recently McCarrison (67), Lucksch (68), Herring (69) and many others. There is no knowledge of direct relationships between the store and the epinephrin output or the metabolism in health or disease, although the literature is full of statements which imply that from the amount of epinephrin in the glands after death (often guessed at by means of the chromium reaction) the degree of efficiency of "adrenal function" during life can be determined.

Relations of the interrenal (cortical) tissue to metabolism. Some observers have maintained that the cortex takes a share in the formation and even in the discharge of epinephrin (70). If this were true a good deal of what has been said on preceding pages would be applicable to the cortex as well as to the medulla. But there is good reason to believe that those who have obtained epinephrin reactions from extracts of cortex have been misled by accidental, in some animals unavoidable contamination with medullary elements. The statement that epinephrin is still given off in detectable amount into the blood after the medulla has been eliminated (70), is demonstrably incorrect (71), (46). The error is based upon misinterpretation of a reaction (dilatation of the pupil of the denervated eye) which, as employed, is far from being an unambiguous reaction for epinephrin.

A striking difference between the cortical and the medullary tissue is that the latter is very richly supplied with nerves, the former much more

sparsely. Not only so, but it has been shown beyond cavil that the output of the active substance from the adrenal chromaphil tissue is strictly controlled by the nervous system, while the cortex or a small portion of it continues to perform its indispensable function, and to perform it faultlessly, when the gland has been denervated as thoroughly as possible. It is worth noting that it is the relatively unimportant part of the organ which is strictly controlled by nerves, whereas the part without which the body cannot live at all exercises its all-important office by direct exchange with the blood. It is of interest to recall that nervous control of the thyroid, the parathyroid, the islets of Langerhans, the interstitial tissue of the gonads, all highly important organs of internal secretion, also remains at present undemonstrated.

Marine (17) and his pupils conclude that the heat production (basal metabolism), as estimated by the respiratory exchange, is measurably increased for a time in many rabbits by adrenalectomy or crippling of the adrenal cortex by freezing. They believe that this depends upon the production of a certain amount of cortical deficiency and that the medulla is not implicated. When the thyroid has been removed the change does not occur, which they think indicates an interrenal-thyroid relationship. In animals that die too soon, within a few days after completion of the adrenal operation, there is a fall instead of a rise in the metabolism, corresponding to what was observed by Aub (72) in cats. They think that Aub's result is due to the fact that cats die too quickly after total adrenalectomy. When partial cortical deficiency is caused a fair proportion of cats show a significant rise in basal metabolism (73). Lack of space prevents further discussion of this matter. Whatever the significance of the changes in the total metabolism may be, they offer no definite clue as to the nature of the profound disturbance which occurs when still more of the interrenal tissue is destroyed.

At this point I hear in imagination the warning of the editor that I "have only a hundred words more." I cheerfully spend a score of them in thanking him in anticipation for his generous forgetfulness of the two thousand by which I have overdrawn my credit. I say nothing of the important relations of the cortex to the sex glands because there are no definite metabolic data; nothing of the meaning of the cortical lipoids, because little is known, unless it be that cholesterin metabolism is not specially controlled by the cortex; nothing of the effect of drugs and bacterial toxins, to which the cortex is so susceptible, because these effects do not prove for it a general "detoxicating function." Finally I omit the supposed intervention of the adrenals in "pigment metabolism" and in the production of immune bodies *sans phrase*.

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LOCAL RESISTANCE AND LOCAL IMMUNITY TO BACTERIA

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It is well recognized that the degree of success obtained by bacteria in invading the animal body depends on the balance struck between two opposing sets of factors; the one inherent in the invading microorganism and the other set inherent in the defensive forces of the host. The factors present in the infective agent are conveniently grouped under the inclusive designation of "virulence" which may be defined as the sum total of the harmful effects in a particular bacterial strain at a given time. These varying forces which fluctuate in the life history of any bacterium, interesting as they are, need not distract us from our main purpose here which is an appraisal of the defensive forces of the animal body and particularly of these forces as they vary under various conditions in different localities of the body.

We know that in order for a bacterium to produce a true infection, local or general, it must penetrate the superficial epithelial covering of its prospective host. This penetration depends not only on the invasive property of the microörganism but also on its localization at a suitable "portal of entry." Only the most virulent and invasive microörganisms can penetrate indifferently at any point through the outer protective epithelium and it is doubtful if successful penetration is possible at any point without some previous abrasion or break in continuity, however small it may be. Once the first barrier has been passed, the second line of defense, which is no longer purely mechanical, involves the active resistance of the body of the host. This resistance is active not only in the sense of the participation of cells located at the portal of entry but frequently of cells that are rapidly mobilized there for the particular purpose of defense.

We recognize clearly that the mechanism of defense against any particular microörganism varies in different parts of the animal body as is evidenced not only by need of a suitable portal of entry, but also by variations in the minimal fatal dose in experimental infections

in accordance with the point of inoculation. In other words, the extent of the infection and the ultimate fate of the animal may depend in a given dose entirely on the area or tissue in which the infective agent is deposited or to which it gains entrance. We recognize, then, local variations in degree of resistance to bacteria and some if not all the factors responsible for the increased resistance of one area over another have been recognized. In some instances mere remoteness from a vulnerable part may account for delayed manifestation of a disease as in the inoculation of rabies on an extremity, but although this remoteness may delay or even modify the manifestations of symptoms, in untreated cases, it serves little to protect. We are thinking more particularly, however, of infections such as those due to the streptococcus where chance invasion or deliberate inoculation may lead on the one hand to a local, recoverable abscess when the portal of entry leads to the subcutaneous connective tissue, or to general septicemia and death when ingress to the general circulation is directly determined or indirectly obtained. The simple fact that the strictly localized infection may or may not extend to a more generalized involvement make us feel certain that we are not in such instances dealing with a strain of the streptococcus with some particular elective affinity, or indeed with a strain with some self-limited pathogenicity, but rather with the more or less successful local reaction of the host which varies remarkably in one part of the body as against another. In general it is true that even temporary limitation of an infecting dose by the structure of the tissue involved determines the outcome. And further, we know that violent local reaction near the portal of entry tends to prevent general involvement, whereas the cryptogenetic infections are the most fatal.

We are now widely informed as to the superior type of generalized protection that is afforded by recovery from many known, or presumably bacterial diseases. In a number of instances we have been able to produce artificially in unaffected individuals not only active immunity of this sort, but even to transfer it for preventive or therapeutic purpose by means of the serum of actively immunized animals. We know furthermore that, at least in the instances of passive antitoxic or antibacterial immunity, protection is due to the presence of antibodies in the serum which have specific effects on the particular microörganism in question.

The polymorphonuclear phagocytes of the circulating blood are, to a large extent, responsible for the conditions of natural resistance

indicated in the preceding paragraph. They are, moreover, certainly coöperative, in all but perhaps pure antitoxic immunity, in the more intense condition of active immunity. The antagonistic properties of the blood fluids (antibodies) are to some extent concerned in natural resistance (immunity) and to a large extent in acquired immunity. But even after admitting a complete coöperation between these two factors on evidence submitted by the proponents of both cellular and humoral theories of immunity there remain unexplained instances of protection against bacterial agents in which no direct participation of either Metchnikoff's phagocytes, in the narrower sense of polymorphonuclear leucocytes, or of the humoral properties of the blood or of both together, can be invoked. There remains a "tertium quid" to explain, for example, natural anthrax immunity, or the marked grade of protection following recovery from typhoid fever.

It is precisely in these debatable and unexplained instances of protection (often of an extreme grade) that we believe that fixed tissue or local immunity is operative and it is our purpose here to sift the evidence for its existence, extent, nature and practical utilization.

EVIDENCE FOR THE EXISTENCE OF A LOCAL FORM OF IMMUNITY. We wish to distinguish here between local variations in natural resistance in the animal body, to the existence of which we have briefly referred, and the reasons for which have to some extent been given, and local immunity, which we define as an acquired, increased protection of some part or tissue superior to that existent elsewhere in the body. This local immunity moreover follows the local administration of the particular microörganism against which protection is evidenced.

A study of the various routes of immunization has been made since the pioneer observations of Pasteur rendered it certain that artificial protection against various bacterial diseases was possible. Indeed the empirical, though happy results of artificial immunization preceding the classic work of Pasteur, in particular the various forms of variolization and vaccination against smallpox, were effected by inoculation in various parts of the body as, for example, the nostrils and the skin. The possible routes of immunization in each particular case have been studied from the point of view of practicability, freedom from untoward results, and the efficiency of the protection ultimately obtained. They have not until recently been studied in relation to the protection of the particular area in which inoculation was practiced. Pasteur found that chickens could be protected against chicken cholera not only by subcutaneous inoculation of B. avisepticus, but by the administration

of the microörganism with the food. He found, however, that the results obtained by feeding anthrax spores to sheep were not as favorable as when vaccines of diminished virulence were administered subcutaneously. Ehrlich found that he could immunize guinea pigs successfully against abrin and ricin by giving these poisons through the mouth and in this way the severe inflammatory reactions produced by subcutaneous inoculation were avoided.

We would further define local immunity as due to a locally superior mechanism for the disposal of the particular microörganism rather than a local mobilization of antibodies generally present in the body. It is evident that satisfactory demonstration of a local as against a general immunity is difficult, owing to the fact that local immunity eventually flows over into general immunity if the process of immunization is continued. Experiments, then, to demonstrate strictly local immunity must be carefully devised. Local immunity may be proved by the local presence of antibodies before their appearance elsewhere in the body or by their local presence in greater concentration. Obviously we are here touching upon the question of the locus or loci of antibody formation. Or again local immunity may be demonstrated, and this we believe most conclusively by the demonstration of a superior method of disposal of bacteria in the particular area in question.

We believe that the local production of antibodies in separately inoculated areas has never been conclusively proved. Wassermann and Citron have given us a very interesting designation for a local immunity based on the supposition that the cells in immunized areas are "retuned" ("Umstimmung") to a greater activity. When these authors injected typhoid bacilli into the pleura or the peritoneal cavity they claimed that exudates from such areas showed higher anti-bacterial property than that of the blood of the same animal. The protocols on which this conclusion is based are in our opinion quite unconvincing. In other experiments they injected bacteria into a rabbit's ear and tested the anti-bacterial properties of the blood at intervals. This power was found to fall when the ear was amputated, which would not in our opinion indicate any more than that the residual antigen, which was continuing to immunize, had been removed. In a similar way the experiments of Roemer on local corneal immunity to abrin, of Von Dungern on the local production of antibodies in crab plasma and of Miyashita on the corneal production of hemolysins did nothing but evidence the localization in these areas of antibodies from the general circulation; in other words, they reflect a general immunity. Hektoen

endeavored to produce hemolysins locally by injecting blood corpuscles of the rat and the goat into the anterior chamber of the eye of dogs but never found antibodies there until after they had appeared in the blood. In similar experiments where the inoculation was made into the pleural cavity the antibodies in the blood also preceded and exceeded their local production. Mutermilch also was convinced that there is no local formation of antibodies to trypanosomes after intrapleural inoculation.

The usual routes of immunization both in man and animals are intravenous or subcutaneous. There is a general impression that subcutaneous inoculation produces a more gradual but more solid active immunity and that the intravenous or intra-peritoneal routes produce more rapid immunity whether active or passive. There remain, however, certain more unusual routes that have been tried in connection with certain projects such as ease of introduction, relatively low toxicity, and recently and most important from the standpoint of strictly local immunity, for the purpose of closing or reënforcing the specific "portal of entry" of the pathogenic microörganism concerned. In this fashion immunization by the mouth, by the trachea and intradermally have been particularly tried. Although attempts at immunization by the mouth extend back at least to the beginning of our formal knowledge of immunity (Pasteur), their significance and importance have only very recently been appreciated. Much of our recent information is more valuable from other viewpoints than as a proof of the existence of a local form of immunity but it is so extensive and so significant that we shall review it in some detail, discussing the results obtained by each of the more important routes of immunization that have been mentioned above.

IMMUNIZATION BY MOUTH. The history of attempts at immunization through the intestinal canal has recently been reviewed in great detail by Calmette. Further reviews, although less complete, have been given by Besredka (1), (2). It seems undoubted that the first experiments in this direction were reported by Pasteur, Roux, and Chamberland in protecting chickens against chicken cholera by ingestion of food stuffs contaminated by B. avisepticus. We have already mentioned this report and also their statement that they were unable to utilize a similar method to any advantage in immunization against anthrax. It should be noted that these experiments were carried out before 1880 and therefore forestall the criticism made two years later by Koch on the Pasteur method of anthrax vaccination which has so widely prevailed. Al-

though Koch admitted that the Pasteur method would protect against artificial infection coming through the skin, he declared it valueless in protecting against the more usual type of accidental infection which is through the intestine.

There followed a series of attempts between the years 1892 and 1903 in the hands of Klemperer, Metchnikoff and others to produce immunity against cholera vibrios and the typhoid bacillus by the ingestion of killed or even living cultures. These experiments are doubtful owing to the fact that some degree of immunity was presupposed when agglutinins were found in the circulating blood of those animals or human beings who had ingested the bacteria. It is now more widely admitted and has been maintained for several years by Gay, Besredka and Calmette among others that antibodies are no measure of protection although they do of course indicate reaction which may or may not run parallel with actual immunity. It is particularly true that a general reaction as evidenced by serum antibodies is no indication of a superior local protection, for example in the intestine, if we admit that this exists. The administration per os of various other organisms such as B. tuberculosis, B. pestis, and B. diphtheriae for immunization has also been studied (Calmette). Although the antibody reaction has often been definitely stated, little accrues from these experiments to our knowledge of local protection although in some instances experimental infection by various routes was attempted and indicates that at least some grade of general immunity can be produced against these bacteria as well.

In the majority of instances these immunization experiments have been carried out with microörganisms whose pathogenic effect is evidenced by their presence in large numbers in the intestinal canal, and increasing information as to the routes of infection make it also likely that the "portal of entry" or locus minoris resistentiae for these organisms is in the intestine. This is certainly true in the typhoidal infections and perhaps also in tuberculosis. The evidences of disease also remain localized largely in the intestinal canal in cholera and dysentery. In other words we believe that it has gradually become more evident that the purpose of immunization by the mouth, if such be possible, would be more to protect certain areas of increased susceptibility by the process of local immunization or to close certain "portals of entry" by the same process. This latter point has been particularly brought into prominence by Besredka.

Coincident with this point of view, which we believe is growing, have been the studies on the enterotropism of certain of these bacteria inaugurated, according to Calmette, by Emmerich. More methodical and conclusive proof has only recently been afforded by Besredka (3) with B. dysenteriae and the typhoid and para-typhoid organisms and by Cantacuzene and Marie and by Masaki with the cholera vibrio.

It is well recognized that generalized infection by the typhoid bacillus cannot be produced in rabbits by ingestion. The resistance to infection in the rabbit as compared with man and the anthropoid apes would be attributed to local factors which we have evidence to believe are present in all animals against those microörganisms which are not normally pathogenic, although present in the intestinal canal. The factors concerned in this normal resistance do not particularly concern us here, but they may be assumed to be due in some part to slow penetration through the intestinal mucosa. Besredka conceived the idea of rendering this penetration more certain in experimental animals by previous injury or actual desquamation of the epithelium produced by the administration of bile. His object in attempting infection in this fashion was not simply to secure infection but also to permit local penetration for the purpose of immunization. He has shown with Basseches that although some degree of protection may be afforded mice against B. para-typhosus B by their ingestion, the immunity is uncertain. He found in short in rabbits (4), (3) that experimental infection can be produced by typhoid and para-typhoid bacilli by bile ingestion followed by the bacteria. Killed bacteria preceded by bile will immunize against subsequent infection by the mouth or intravenously. In the latter case protection would likewise be due to local protection in the intestine as the bacteria are enterotropic. The greater susceptibility to infection produced by bile has been confirmed by Zingher and Soletsky, by Neri and by Glottoff. The first two of these observers, however, do not admit the possibility of immunization by this method. Webster in a similar set of experiments likewise admits that bile lowers the resistance of mice to para-typhoid infection but states that the dose necessary is near the limit of toxicity of this substance.

In experiments with the dysentery bacillus (Shiga) Besredka (5) has shown that no bile is necessary to promote penetration either for infection or immunization and the latter can be produced by giving killed cultures per os. In this as well as in the typhoid experiments there is indirect evidence that the protection is local in that antibodies are not

found in the general circulation. Masaki, in the experiments to which we have already referred, finds that rabbits are protected against cholera by bile sensitization followed by killed or living vibrios. There is, to be sure, the formation of some antibodies but the serum is not protective, although the animals themselves are protected against infection by any route. In all these experiments where protection is tested, whether by intestinal infection or by intravenous inoculation we may regard the protection as local whether the infecting dose be applied to the protected area or eliminated through it following entrance elsewhere.

It would seem then clear that bile may increase the pathogenicity for the intestine of certain microörganisms and may also increase the penetration of killed or living cultures of these bacteria for immunizing purposes. The latter point is still disputed. Such protection, if it exists, would seem to be local rather than general.

Besredka has by no means allowed the matter to rest with these undoubtedly interesting and to some extent accepted experimental facts. Owing to his belief in the essential value of intestinal immunization preceded by bile several sets of results have already been presented as to the possibility of application of this method to human beings. The report of Nicolle and Conseil (1), (2) does no more than point to the experimental possibilities of using this method in man in protecting against malta fever and dysentery. Vaillant in an outbreak of typhoid in Pas de Calais gave Besredka's sensitized vaccine either by mouth, preceded by bile, or subcutaneously. In 1236 subjects vaccinated by mouth there were only two cases of typhoid (0.17 per cent); in 173 who were vaccinated subcutaneously there were four cases (2.3 per cent) whereas among over 600 unvaccinated there were fifty cases of typhoid or 7.7 per cent. In a later report Besredka (10) gives the results of immunizing students in a military academy where 43 cases of typhoid had occurred within ten days in 1923. Two-hundred and fifty-three students were vaccinated by subcutaneous inoculation and 268 were given killed organisms preceded by bile by mouth. Among the first there were ten cases of typhoid whereas only five occurred among the latter. In both instances they occurred within ten days after immunization and were mild.

We are informed by Calmette that experiments on the possibility of immunizing human beings against B. dysenteriae and also the cholera vibrio are actually being undertaken, but no information as to their efficacy is as yet available.

Experiments on immunizing cattle against tuberculosis by the intestinal route have been under way for a number of years by Calmette and Guerin (Ann. Pasteur 1906–1922). Although they are encouraging, no certain practical results can as yet be claimed for either man or animals. The reports on intestinal immunization with other bacteria, such as streptococcus, B. pestis and the like are of doubtful significance.

CUTI-IMMUNITY. The results obtained in immunization of the skin seem distinctly more interesting as indicating a strictly local form of immunity, and as bearing on the alleged protection of a specific portal of entry or of an area of greater susceptibility.

Besredka (1) has pointed out that vaccination against smallpox is a true cuti-immunity. This disease in common with several others due to filtrable viruses is recognized as dermotropic under both experimental and accidental conditions of infection. It may be doubted whether the skin represents the usual portal of entry in variola, but certainly immunization of the skin protects against infection and presumably without the presence of antibodies for any length of time. Besredka claims on the basis of experiments in guinea pigs that skin vaccination protects against infection on the skin elsewhere in a very short time, but similar initial inoculation into the peritoneum avoiding the skin does not protect against secondary skin infection. In both instances antibodies are produced but they disappear before the skin protection is lost and immune serum from the rabbit, although it neutralizes the vaccine outside the body does not prevent its producing a lesion when given simultaneously in the inoculated guinea pig.

The evidence for a local type of immunity to the streptococcus is more voluminous and conclusive. Fehleisen not only isolated the streptococcus for the first time from lesions of human erysipelas but was able to reproduce the disease with pure cultures both in man and animals. On reinjecting two of the first positive inoculations which he made in human beings from three to four weeks subsequently, no erysipelas followed and yet in the second of these cases the first inoculation had not only been positive but had followed a spontaneous erysipelas which had occurred about ten months previously.

These observations, limited as they were, certainly strongly suggested a transitory immunity and were so accepted by Fehleisen and later by Metchnikoff (2). They were, however, not regarded as indicative of active immunity by Koch and Petruschky in the light of their own experiments. The first complete experimental study of this condition of streptococcus immunity was, we believe, made by Meierowitsch in

1887, who found that healed erysipelas in rabbits protects against new inoculation for a period of from 1 to 2 months. Similar observations were made by Roger a few years later, and he makes the definite statement that not only is the inoculated ear protected, but likewise the other ear. These studies were followed by similar work by Gramakowsky, who further makes the interesting observation that rabbits that have become insusceptible to superficial skin inoculation do not always withstand a fatal intraperitoneal dose. Denys and Leclef produced erysipelas with very small amounts of a broth culture of some of the strains they employed and found recovery was followed by little or no reaction on second inoculation. Subsequent work by Cobbett and Melsome is interesting in this connection. They failed to obtain erysipelas on reinoculation in the same ear during a period roughly of from 22 to 100 days following the first successful inoculation. They also found that there is some resistance when the second inoculation is given in the other ear, although it is not so marked as when given in the first ear. They interpreted this partial protection of the other ear as due to a condition of general immunity as against the purely local immunity present in the first ear. Cobbett and Melsome's work likewise indicates that a nonspecific inflammation such as that produced by mustard oil will prevent successful inoculation.

These observations led recently to a restudy of these conditions by Gay and Rhodes who corroborated the facts of others that recovery from experimental erysipelas in rabbits is followed by a complete protection of the point of inoculation. The fatal intravenous dose of the particular strain of streptococcus they employed and the symptomatic intradermal dose were the same. Complete protection against intradermal inoculation by previous streptococcus infection or immunization frequently does not protect against intravenous infection. The reverse set of conditions apparently also prevails. That is, intradermal immunization in the same dosage and at the same intervals protects better against intradermal infection than does intravenous immunization in the same dosage and at the same intervals.

It may well be that whatever success attends Wright's method of vaccinotherapy is due to cuti-immunity as Besredka has suggested (1), (6), (7). At all events Wright's method has seemed to work best with localized streptococcus and staphylococcus infections. Levaditi has recently made some interesting observations in this connection and found that war wounds infected with streptococcus tend to clear up at irregular intervals. In a wound from which the microörganisms have

disappeared there is found the property of destroying rapidly considerable numbers of the streptococci that had previously infected it; when the infected bandages from another wound in the same individual that has not become sterile are applied to the wound that has become sterile the bacteria disappear rapidly. Wassermann and Lederman some years ago suggested the application of a killed staphylococcus culture or of a staphylococcus salve to prevent further spread of infections. Besredka (8), (6), (7) has taken this question up anew with interesting results. He finds when the filtrates of staphylococcus or streptococcus are applied by rubbing or by compress to certain areas of the skin of guinea pigs that within twenty-four hours such areas are protected against local infection by this same organism. These experiments have been repeated by Rivalier in the rabbit with similar results; intraperitoneal injection did not protect at all. Bass in a limited set of experiments on human beings claims to have obtained results in furunculosis and other local infections. These results have recently been claimed by Gratia to be due to the broth alone used in the culture media, the significance of which will be evident in our later discussion on clasmatocytes.

Recent reports on immunization against anthrax afford in many respects the most interesting contribution to a strictly local immunity. Besredka (1), (9) has reported on the difficulty of protecting laboratory animals against anthrax by the ordinary Pasteur method which is so successful in larger mammals. He found that the first vaccine may be rubbed into the shaved skin of guinea pigs with a resultant local inflammation; if the second vaccine is used in this way it produces a fatal generalized infection. The second vaccine may be used, however, after recovery from the first local reaction. Such doubly treated guinea pigs are then protected against virulent cultures. Rabbits similarly vaccinated are protected against infection by any route and the virulent culture may be applied locally from the first. Intracutaneous injections may be used instead of inunction. The surprising part of the experiments is that fatal intradermal doses administered to either animal intraperitoneally, intravenously or intratracheally are harmless if the skin is not contaminated. Noetzel, in an article to which we shall later refer, had noted as early as 1898 that rabbits will withstand 200 times the fatal subcutaneous dose of anthrax bacilli when the injection is practiced intraperitoneally without skin injury. The fatality then would depend not on the septicemia primarily but the infected skin. Furthermore intraperitoneal injection does not protect animals as well

as does the intracutaneous route of immunization. It is found, moreover, that the serum of animals immunized by the skin does not protect against anthrax infection.

These experiments of Besredka are surprising but at all events can be interpreted it would seem only in the light of a specific local immunization of the portal of entry. The experiments have recently been confirmed in detail by Combiescu and by Mazucchi. They have moreover been extended by similar observations in horses by Brocq-Rousseu and Urbain.

The possibilities of immunization by the respiratory tract have long been considered. Dsergowsky and Blumenau claim to have immunized children to diphtheria by applications of diphtheria toxin to the nasal mucosa. Besredka (10) has considered the matter more systematically by intratracheal injections of various bacteria in rabbits. Animals are more resistant to B. paratyphosus, for instance, when the culture is given by this route than when given intravenously. This resistance can be decreased by bile. Recovery affords protection. Besredka's first impression was that this form of inoculation affords a protection that is both local and general. The generalized nature of the immunity is evidenced by the antibodies.

This line of experimentation has been corroborated by several observers. Cecil and Steffens find, for example, that monkeys can be protected against the pneumococcus by intratracheal injections of vaccine. Pfenninger, D'Aunoy and Clark have shown that antibodies may readily be produced against various antigens by the intratracheal route. There remains, however, in our opinion, no evidence from these results, interesting as they are, to show that the immunity is local rather than general.

It should be pointed out here that close parallelism which exists between conditions of immunity and conditions of hypersusceptibility or anaphylaxis, is likewise exemplified in their respective local phenomena. Just as there is a local as contrasted with a general immunity so the phenomena of hypersensibility may be characteristically local with little or no generalized hypersusceptibility or the reverse. Zinsser has shown that conditions which produce generalized tuberculin hypersusceptibility are quite different from those which produce cutaneous hypersusceptibility. Mondolfo and Coscera have shown that the skin test for tuberculosis is most intense over the area of infection. Kohler and Heilman have shown that greater skin sensitivity to rabbit serum is produced in man by sensitization that is produced intradermally than when the first injection is given intravenously.

THE DISPOSAL OF BACTERIA IN THE RESISTANT AND IN THE IMMUNE ANIMAL. We have already referred to the two main factors that have been recognized as responsible for the disposal of bacteria under conditions of natural and acquired immunity—namely polymorphonuclear leucocytes and antibodies. These two factors are operative both locally and generally in the animal body. We have further indicated that not all instances of protection are explicable on the basis of these two factors alone. We have suggested that natural resistance is due largely to the activity of phagocytes aided under certain conditions by the adjuvant action of the blood fluids (opsonic effect). The marked increase of protection in acquired immunity is paralleled by increase in antibodies, all of which have the property of uniting with the antigen and rendering it more susceptible to phagocytosis. There is an increasing impression (Zinsser) that there is a single antibody in immunity with a single essential property of combination with (sensitization) the antigen, but which may also manifest itself otherwise in various ways (agglutination, precipitation, opsonization, lysis, etc.) in accordance with the physical conditions that prevail.

The polymorphonuclear phagocytes are neither increased nor have they essentially increased properties of phagocytosis under conditions of acquired immunity, and yet we believe that both resistance and immunity are primarily and ultimately cellular processes; primarily in the sense that antibodies are recognized to be formed in the cells (Madsen), and ultimately in view of the fact that phagocytosis is the usual and perhaps the inevitable expression of the destruction of bacteria in the body. We need not regard the Pfeiffer phenomenon of extra-cellular bacteriolysis, particularly as carried out in the test tube, as invalidating the importance of phagocytosis. Metchnikoff (3), to whom we owe our appreciation of the importance of phagocytosis, stressed the importance of the *polymorphonuclear* cells or microphages in the majority of bacterial infections. The response of these cells in an infection is obvious and purposeful. They collect locally in areas of inflammation, increase in the general circulation and can be actually seen to engulf and digest bacteria. The accumulation of microphages in large numbers is usually of happy prognostic significance, their absence is unfavorable. In short, the evidence for the importance of the polymorphonuclear cells in protection is overwhelming; it has become increasingly evident, however, that these particular cells do not account for the entire sequence of events in protection against bacteria.

The importance of the humoral factors of immunity can readily be harmonized with phagocytosis by microphages; there is not, however, sufficient evidence, we believe, to indicate that these cells are the source of antibody formation as Metchnikoff was inclined to urge. For this primary and essential phase in the development of acquired immunity we are forced, by exclusion, to turn with more attention to other cells of the animal body. And in seeking for the locus of antibody formation we are influenced by the ingenious receptor hypothesis of Ehrlich to presuppose that the cells that form antibodies are the cells which normally dispose of the bacteria to which such antibodies are formed. There have been many objections to this "receptor" hypothesis even in this, its most fundamental conception. But at all events, the latest work on the locus of antibody formation and on the disposal of bacteria has brought into prominence cells other than Metchnikoff's microphages which we are now brought to consider in some detail in the light of our present inquiry.

It was Metchnikoff's (2) own work which first pointed out and designated important phagocytic cells other than the polymorphonuclear leucocytes. In this author's early work on experimental erysipelas he mentioned the constant presence of large mononuclear cells to which he gave the name of "macrophages." Metchnikoff expressly claimed that these cells had nothing to do with the disposal of the streptococcus which produces erysipelas and the entire subsequent work of Metchnikoff (3) and his pupils tended to associate these cells with a type of phagocytosis which only indirectly involves the agents of acute infection. It was agreed that the macrophages dispose of animal cells such as protozoa and red blood corpuscles and even the polymorphonuclear cells; and further, such polymorphonuclears may have already ingested bacteria. It is further admitted that they are responsible for phagocytosis of certain more "chronic" bacterial infectious agents such as the tubercle bacillus and the bacillus of leprosy. Recent work tends to define more clearly what cells may be understood by "macrophages" and perhaps to attribute to them, in re-designation, a greater responsibility in the disposal of bacteria in general.

Metchnikoff, impressed with the importance of the cell contribution from the circulating blood in acute inflammatory processes, would apparently rank the large mononuclear cells of the blood as the macrophages "par excellence" and undoubtedly attributed less importance to similar cells normally present everywhere in the connective tissue and serous exudates, to say nothing of the endothelium which may be

the common origin of both of these cells. Too little importance has probably been paid to the rôle of the "histogenous leucocytes" in inflammation, that is to say in conditions of normal resistance to bacteria. Adami in his classical treatise on "Inflammation" as well as in his text book on *General pathology* has emphasized this phase of the question. The importance of so-called "endothelial" cells has been particularly studied in inflammation of the peritoneum. We shall later discuss the designation of the cells but may here refer to the significant observations that have been made.

Cornil and Ranvier called attention to the very active proliferation of "endothelium" in the great omentum produced by injections of dilute nitrate of silver. Issaef thought cholera immune serum prevents an otherwise fatal peritoneal infection in guinea pigs by suspending the initial leucopenia and increasing the proportion of "round cells." Durham, 1897, found that these cells appear in the exudate as early as the 15th hour in guinea pigs that have been given sublethal injections of several bacteria and that they are markedly phagocytic. They predominate over the microphages after the first 24 hours but are absent in fatal infections. Both Jensen and Noetzel likewise pointed to the importance of the extensive endothelial lining of the peritoneal activity in its relative resistance to streptococcus. Wallgren thought direct endothelial phagocytosis of great importance in this connection. MacCallum later refers to the important phagocytic properties of both the serosal cells and the endothelium of the lymphatic vessels which drain the peritoneum and his work with pigment granules has been extended and confirmed by Cunningham (1), (2). It should further be noted that the great properties of resorption, both of pigment and of bacteria, from the peritoneum are directly related to this type of phagocytosis. This rapid resorption further leads to rapid dissemination of bacteria from the peritoneum to all parts of the body (Jensen) and destruction of bacteria is by no means confined to the original site of infection.

The observations on peritoneal phagocytosis were later continued by Beattie 1903 who regarded the large mononuclear macrophages as derived largely from the omental endothelium. An extended series of valuable observations by Buxton and Torrey extends and reiterates the importance of macrophages in the disposal of bacteria and pigment injected into the peritoneal cavity of rabbits. In pigment injections (1) the macrophages which predominate at first take it up. Later the microphages participate for the first twenty-four hours, but are them-

selves engulfed by the macrophages which begin to increase at the end of this period. In continuation (2) they followed the fate of typhoid bacilli given in sublethal doses. There is an explosive decrease of bacteria in the peritoneum in the early hours and the rapid appearance of them in various organs. An increase may then take place in the peritoneum and later a second sharp decrease both there and in the liver occurs. When the factors responsible for the diminution of typhoid bacilli are studied it is found (3) that the bacteria appear rapidly in the mediastinal lymph nodes where they are phagocyted almost entirely by the macrophages. The importance of the omentum in peritoneal inflammation, to which previous observers had drawn attention, was then studied following both pigment (4) and typhoid bacilli (5), and here again the macrophages are the more important phagocytes; microphages appear however to be important adjuvants. Buxton and Torrey also came to a conclusion in reference to these tissues (omentum, lymph nodes) concerning the nature of peritoneal macrophages which is of importance in view of the later observations on these cells. They regard the large mononuclear phagocytes not as directly endothelial in origin, but refer to them as "trailers" which they would identify rather with Ranvier's "clasmatocytes" which are supposedly derived primarily from connective tissue. In a further contribution (6) they point again to the macrophages as responsible for the acquired immunity of the rabbit to typhoid bacilli. This has recently been confirmed for passive antistreptococcus immunity by Kanai. Widal, Ravaut and Dopter have also assigned important phagocytic functions in bacterial infections to the "endothelial" cells in exudates.

There has been much interest on the part of bacteriologists and of surgeons in the difference in susceptibility to bacterial infections between the pleural and peritoneal cavities. It is generally recognized (Noetzel (2), Yates, Gay and Morrison) that the peritoneum is less susceptible than the pleura. Several writers have considered the factors involved in pleural infection experimentally (Kracht, Grober, Noetzel (2), Landsberg, Gay and Morrison). It is agreed that resorption takes place rapidly from the pleura at first as well as from the peritoneum (Grober; Noetzel (2)). Fluid, however, tends to accumulate in the pleura due to difference in pressure as compared with the peritoneum and to plugging of the stomata or to pressure on lymph channels (Grober). The absence or presence of a proteolytic ferment may be the decisive factor (Landsberg). At all events the peritoneum surpasses the pleura in area of lining cells whether for facilitation of resorption

or for actual phagocytosis. Several authors hint at the importance of the nature of exudate itself but little attention has been paid to this aspect in the pleura as compared with the peritoneum until the work of Gay and Morrison. We prefer to consider later the importance which these authors would assign to the presence of tissue macrophages or clasmatocytes.

Another series of observations points to the importance of the vascular endothelium, and particularly those modified endothelial cells of the liver known as Küpfer's cells, in phagocytosis. Werigo found that when anthrax bacilli are given intravenously in rabbits they are largely disposed of by the hepatic endothelium which assumes bizarre and multinucleated forms under the stress of extreme phagocytosis. Adami, Abbott and Nicholson found a similar fate awaiting colon bacilli. Kyes has recently again drawn attention to Küpfer's cells (hemophages) in the liver and to their analogues in the spleen in disposing, not only of red blood cells, but also of pneumococci in the pigeon which is highly resistant to this microörganism. Nagao has also shown that liver endothelium is the most important place for the disposal of streptococci in guinea pigs. Hopkins and Parker had indicated the importance of cells in disposing of streptococci in the lungs, but Nagao would regard the destruction in this locality as due largely to polymorphonuclears.

The importance of fixed tissue phagocytosis of this type has been extended by Motohashi (1), (2) and Cary has given reasonable grounds for supposing these cells to be responsible for hemolysin formation. As a continuation of this line of thought Thomson on the supposition that tuberculin stimulates macrophage activity, injected small amounts of this substance coincidently with red blood corpuscles and obtained a greater antibody output. Manwaring and Fritschen by a novel perfusion method have shown that tissue affinity for various bacteria is normally large in the liver and is markedly increased there as a result of immunization. In this connection there should also perhaps be noted the observations of Bull on the importance of agglutination in the disposal of bacteria in the body of immunized animals. In a series of papers (1), (2), (3), (4), (5), (6), (7) this author has traced the well-known temporary or permanent disappearance of bacteria from the blood stream of both normal and immune animals. He has shown that agglutination occurs in the animal body even when absent in the test tube and would attribute the decrease of bacteria in the peripheral stream as due to clumping in the capillaries. In this locality, destruction of the bacteria then takes place readily, and although Bull

attributes this destruction largely to polymorphonuclear phagocytes his observations, it seems to us, do not rule out but make extremely probable an even more important endothelial phagocytosis.

Here also should be recalled two striking relations that have been pointed out between bacterial infections and macrophage response. Mallory's macrophage (endothelial) response to infections with the typhoid bacillus is a well known and pathognomonic feature of this disease. Whether this response is protective or not as Mallory believes requires we believe further proof, much as we should prefer to agree with it. The epithelioid cells of the tubercle are also undoubtedly macrophages as indicated by Borrell and as the work of Evans, Bowman and Winternitz conclusively shows. Here again the protective nature of the response can only be suggested.

This summary so far has evidenced that there are cells of the "macrophage" type, using this term in its most inclusive sense, in the capillary endothelium and in serous exudates which have distinct phagocytic properties. There are two points that are not as yet clear. What is the relation, if any, of these cells to each other in the generic sense, and what bearing do they have on localized immunity? Both questions may be approached by considering yet another area in which bacterial destruction by macrophages occurs, namely, the subcutaneous connective tissue. Macrophages are recognized as appearing in the subacute stage of any localized inflammation particularly when it is in process of healing (Adami). This appearance of macrophages has been particularly noted in localized streptococcus infections (Metchnikoff (2); MacCallum (2); Roger (2)). Levaditi has not only noticed a localized immunity in healing streptococcus wounds but has associated macrophages with this condition. A series of other observations associate macrophages with streptococcus infections in various parts of the body and particularly infections in which an active resistance is being manifested (Rosenow and Ashby; Cecil; Sampson, Kerr and Simpson). In cultures from living tissue grown in vitro Rous and Jones (1), (2), (3) found that macrophages, apparently derived from endothelium, had marked phagocytic power for bacteria whereas fibroblasts had no such power. In subsequent experiments Smith, Willis and Lewis show that macrophages, designated by vital staining criteria as "clasmatocytes," in tissue cultures have the greatest phagocytic properties.

Recent definition of macrophages. Recent work by histologists in particular has led to a clear definition and identification of a group of cells occurring primarily in connective tissue but also else-

where in the body, to which various names have been given: "clasmatocytes" by Ranvier, "ruhende Wanderzellen" by Maximow, and "tissue macrophages" by Evans and Scott. The interest of histologists in this group of cells has been primarily to throw light on the origin and relationship of "cell stems" (Sabin). Certain characteristic properties of the clasmatocytes or macrophages have been pointed out and have given the criteria of their differentiation. Clasmatocytes may be differentiated from the fibroblasts of connective tissue by their properties of taking up and retaining in their vacuoles certain dyestuffs, particularly those of the acid azo group (Evans and Scott). The clasmatocytes as they occur in connective tissue usually have definite pseudopodial processes and have been referred to as "trailers" (Buxton and Torrey (4)). When they occur in exudates in serous cavities they are usually ovoid in appearance, with a large excentric and often indented nucleus, which latter form resembles the transitional cells of the circulating blood.

We have already referred to the fact that these clasmatocytes as they occur in serous exudates have often been regarded as endothelial or serosal cells, and yet Ranvier (2) states clearly that they are probably the same as connective tissue clasmatocytes and Marchand agrees to this. Buxton and Torrey (4) also insist that these cells, free in the peritoneal cavity, are not serosal in origin but derived from the connective tissue, a fact which has now been conclusively proved with proper differential staining by the work of Schott and of Cunningham (3), (4) and of Gay and Morrison.

The precise origin of clasmatocytes or "histocytes" as they are also called, has been a matter of much consideration but of no final decision by histologists. Sabin, who has made a most careful review of the subject, tells us that clasmatocytes are at least allied to the large mononuclear cells and the transitionals of the circulating blood. They are certainly of a common origin with these cells from the embryonic endothelium and may actually be derived from the adult endothelium, as Mallory, Foot, Permar (1), (2), (3), (4), (5) and Haythorn have insisted.

The precise relation of the monocytes (large mononuclears) of the circulating blood to the tissue macrophages or clasmatocytes is not fully understood. It has been claimed by several, notably McJunkin, that they are identical. A recently critical study by Simpson would seem most informative; she concludes that "if there is a conversion of monocytes into macrophages (clasmatocytes) it is limited in time and

space and does not occur indiscriminately in the general circulation" but she further concludes that there is a close biological relationship between the two types of cells.

Endothelial cells have undoubted phagocytic power both for pigment and for bacteria as we have exemplified from the literature in the previous section. Clasmatocytes, using this term to include wandering tissue macrophages and the cells of serous exudates, are agreed to have an even superior ability at least for dyes. They have in addition migratory properties, less to be sure than the polymorphonuclear leucocytes, but superior to the fixed tissue endothelium. The clasmatocytes moreover have a superior property in resisting extraneous influences (Lewis and McCoy) which would make them valuable agents in protecting the body against bacteria.

To summarize to the present point: Metchnikoff's mononuclear phagocytes (macrophages) include monocytes of the circulating blood, endothelium of blood vessels, the cells of serous exudates, and wandering macrophages or clasmatocytes of the connective tissue. These cells are closely related histogenetically and also by their ability to take up certain dyes or pigment particles. This phagocytic ability for dyes is to some extent paralleled by an ability to engulf bacteria and thereby indicates their importance in protection against infection. It is suggested at least that the importance of these cells in accounting for the disposal of acute bacterial agents of infection has been under-estimated. In the case of the endothelium at least bacterial disposal and even antibody formation have been demonstrated. Similar and probably more important destructive properties for bacteria are proved to exist in the so-called "endothelial" cells of serous exudates. These cells, however, are probably clasmatocytes or wandering tissue macrophages and apart from their vicarious functions in serous cavities very insufficient though suggestive evidence exists for their bactericidal function in their normal positions. There is evidence to suggest that the clasmatocytes may be the bacterial macrophages par excellence.

A study of this function of clasmatocytes in the disposal of bacteria has recently been made by Gay and Morrison. These authors have continued their work on localized streptococcus infection with increasing interest in the clasmatocytes as identified by modern histological methods. They have confirmed evidence which shows that the cells normally present in the pleural and peritoneal cavities are clasmatocytes and not serosal cells. Moreover they have shown that the outcome of experimental infection of these cavities by the streptococcus depends

on the number of the clasmatocytes present during the infection rather than the number of polymorphonuclear cells. They have given methods of increasing one or the other type of cell. Broth injection into the pleura in particular attracts clasmatocytes and protects against infection. Gratia also has shown that broth will protect localized areas of the skin against streptococcus and would explain the recent experiments of Besredka and Urbain (8) in this way.

This work of Gay and Morrison has dealt largely with induced variations in natural resistance but preliminary experiments indicate that local immunity both active and passive may also depend on the presence or mobilization of clasmatocytes. It is further clear from the work of these authors that the variations in resistance in different tissues or areas of the body in respect to their strain of streptococcus are associated with the relative numbers of clasmatocytes actually present or accessible in such areas.

We have gone thus fully into consideration of clasmatocytes and endothelium as to some extent at least responsible for natural resistance and acquired immunity to bacteria, because this factor offers the best explanation of local resistance and local immunity. That such a variation in the protection of one part or tissue of the body as against another really exists both naturally and artificially (immunization) we hold as demonstrated from the evidence we have submitted in the first part of this review. Further advances in the recognition of the importance of local immunity and its utilization in the prevention and treatment of bacterial infection will depend in large part on understanding more fully the mechanism involved.

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AURICULAR FIBRILLATION

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Our knowledge of fibrillary contraction of heart muscle as produced experimentally, begins with the report of Hoffa and Ludwig (1850). Ventricular fibrillation was produced by them in cold-blooded and mammalian hearts by electrical stimulation, strong, rapidly repeated shocks being delivered by a "Rotations-machine" in an endeavor to produce tetanus. They described a limited region of "tetanus," or rather a condition of persisting contraction immediately about the electrodes; but in the rest of the heart contractions were recorded which were weak and absolutely irregular. Muscular elements no longer contracted synchronously, so that certain elements were relaxing while others were contracting; thus resulted a contractile hodge-podge ("wirrwarr") involving both intensity and rhythm, a condition which persisted after the stimulation ceased and of which they spoke as "Flimmern." They recorded as high as 604 contractions per minute from the rabbit's ventricle, but these were so weak and ineffective that dilatation supervened. Subsequent recovery, with normal beats, was noted, as well as the fact that ventricular fibrillation of the rabbit's heart was not stopped by vagus stimulation. Einbrodt's description (1874) is essentially that of Ludwig in whose laboratory he worked.

Vulpian in 1874 describes the effects of direct faradization of the ventricles of the dog. When electrodes, 5 or 6 mm. apart, are placed on the ventricle and strong stimuli are used, the normal systole ceases and is replaced by an irregular, incoherent, tremulous movement of the whole musculature, which then goes over into fibrillary contractions ("frémissement fibrillaire," "mouvement fibrillaire"). Thus after Vulpian, we apply the term "fibrillation" to these incoördinated contractions produced by faradizing the heart muscle or otherwise arising. He noted the fact that while localized stimulation anywhere threw the whole ventricular musculature into fibrillation, the incoordination did not extend to the auricles which continued to contract rhythmically even long after the ventricles had completely lost their excitability subsequent to fibrillation. Vulpian also was the first to

note that local faradization of the auricles abolishes normal systole, which is replaced by a strong, incoördinate flutter (tremblotement); that the incoördination does not tend to persist after stimulation ceases, but that a rest period, later described by Gewin as the "post-undulatory pause," supervenes and that regular rhythmic contractions then appear. Generally speaking, then, fibrillation of the mammalian auricle tends to be a much more transitory phenomenon than that of the ventricle.

On the basis of the descriptions of these earlier workers, supplemented by the more comprehensive analytical studies of recent workers, the general process of fibrillary contraction of heart muscle may be described as an incoördinate, disorderly and extremely bizarre contractile process in which normal systole and diastole no longer occur, the impression being given that individual fibers or groups of fibers are contracting independently (hence the name fibrillation). While certain regions of the fibrillating tissue are at rest, other adjacent areas or areas widely separated from each other may show synchronous contraction. The surface of the fibrillating chamber shows areas of fine twitchings, of flickering and tremulous movements, combined with coarser undulating waves of muscular contraction which progress slowly through the muscle mass, moving now in one direction, now in another, being continually blocked in their progress by interference with other waves. In one and the same heart, every gradation may at times be seen, from the condition in which the coarse undulatory movements predominate, a condition to which Kronecker (1896) referred as a rolling movement which is comparable to a stormy peristalsis, to that in which the fibrillating tissue presents the appearance of a quivering mass or shows coarse, rapid, fluttering movements with superimposed fine, fibrillary twitchings (Robinson, 1913). Riotous and chaotic as this fibrillation appears to be, analysis indicates that it may be aptly spoken of as a contractile *maelstrom*, for it appears that contractions are not independent of each other, but that the contractile impulse travels in a ringlike circuit repeatedly returning to and involving a given region after completion of each circuit (Garrey, Mines). This idea of a trapped wave, as presented by Garrey and by Mines, has been generally accepted by present day experimenters (c.f. *infra*).

The complexity and variability in the appearance of fibrillating cardiac muscle is indicated by the great variety of the terms under which it has been described, among which are the following: fibrillation,

delirium cordis (Ludwig), intervermiform movement (Mills), fibrillary twitchings, contraction fibrillaire, trémulations, frémissement fibrillaire (Vulpian), mouvements trémulatoires and mouvement fibrillaire, tremblotements musculaires, undulatory movements (Gaskell), Ondulation, Wühlen und Wogen, Flimmern (Hoffa and Ludwig), Herz-zittern (Einbrodt, Kronecker), circulating rhythm (Mines) and circus contractions (Garrey). We are beginning to appreciate, furthermore, that several clinical conditions may be very closely allied, in fact may represent only quantitative variations of the same process, namely, flutter (MacWilliams; Jolly and Ritchie), paroxysmal tachycardia, auricular tachysystole (Rihl, Rothberger), auricular and ventricular extrasystoles of certain types. The identities and relationships in the conditions described under these various names can be decided only after consideration of the nature and mechanism of fibrillation. But certain apparent differences and their explanation may be considered at this time and serve to make easy a realization that all the phenomena are explicable by a common mechanism, that of *"circus contraction."*

IDENTITY OF THE FIBRILLARY PROCESSES. Early investigators were generally inclined to consider the fibrillation of the mammalian heart as something set apart and fundamentally different from the undulatory incoördination which we now call fibrillation of the cold-blooded heart; in fact, they carry the differentiation into the mammalian field, considering auricular fibrillation different from ventricular fibrillation, and even subdividing the character of ventricular fibrillation, chiefly on the basis of differences in the tendency to spontaneous recovery. Spontaneous recovery of auricles from the fibrillary state is generally reported to be the rule in all animals, although this tendency is far more marked among some groups than among others. In general, spontaneous recovery of the auricles is universal in the cold-blooded animals most commonly worked with, viz., the land and fresh-water turtles and the frog. From our own experience we can add to this class the Selachians in general, the alligator, and the marine turtles, in all of which there is practically always recovery from auricular fibrillation with subsequent return to rhythmic beats.

In the warm-blooded mammals we find marked individual differences. Recovery of the fibrillating auricles of the cat and rabbit, for example, is so facile that it is difficult or impossible to keep them in the fibrillary state for the purposes of experimentation. In the dog, recovery of the auricles from fibrillation is practically always to be expected,

sometimes immediately after the inducing agency has been withdrawn; on the other hand, it may persist for hours. A report of Th. Lewis (1912) shows chronic auricular fibrillation to be the cause of *pulsus irregularis perpetuus* in the case of a horse. In man the clinical findings on the one hand indicate paroxysmal auricular fibrillation lasting for periods of only a few minutes (Popper), for hours or for days, but clearing up and recurring (Mackenzie, Hewlett, Hering, Wenckebach). On the other hand, we have reports of its persistence for fifteen years (Bishop, Coffen), in one report for thirty-two years (Heitz), or even permanently.

Similarly in the case of the ventricles of mammalian hearts, "recovery occurs with different degrees of facility, in the dog with much difficulty and usually only after fibrillar contraction has lasted for a considerable space of time." "In young mammals, foetal or after birth, recovery (of the ventricles) seems to be the rule" (MacWilliam), and in a large number of instances MacWilliam saw recovery in the rat, cat, mouse, hedgehog and fowl. This is the common experience of experimenters in this field. Porter goes to some length to show that the difference in the tendency to recovery from fibrillation is not fundamental, but merely a matter of degree. Porter further stated that there is no foundation for the view that fibrillary contractions caused by electrical stimulation are different from those produced by mechanical injury or shutting off the blood supply. Certain workers, notably Kronecker, Tigerstedt and Michaelis, have maintained that the fibrillating ventricle of the dog will not spontaneously recover its ability to beat normally; but the idea is obsolete. It cannot be supported in the face of the positive results of the many investigators who do record spontaneous recovery (MacWilliam, Gley, Porter, Fischel, Garrey, Robinson, Levy, Lewis). Some of these authors found that fibrillation disappeared after cooling. Porter restored normal beats by first cooling and then perfusing with warm oxygenated blood or Ringer's solution either through the coronary system or the vessels of Thebesius. Magrath and Kennedy restored normal contractions by simply reëstablishing coronary circulation under proper conditions of oxygen supply and blood pressure. MacWilliam (1918) states that for years by appropriate use of drugs or chemicals, he has not failed to stop fibrillation in a single instance (experimentally). Erlanger frequently was unable to stop the fibrillating calf's heart, even by perfusion by Hering's method with 1 per cent potassium chloride; when contractility returned, fibrillation again set in. These differences do not, however,

argue against the identity of the processes involved. The fibrillary undulations of the cold-blooded heart change to fine fibrillation like that of the mammalian heart when warmed to 30°C. (Bätke, Gewin); on the other hand, the warm-blooded heart, when cooled, changes its wild delirium to a type in which slow peristaltic undulatory waves course over the tissue as in the cold-blooded heart, a type which is identical in character with that which Porter found following perfusion.

These facts support the contention of Langendorff that the phenomena in cold- and warm-blooded hearts are not essentially different. There is little difference in the tendency of large hearts of warm-blooded and cold-blooded animals to fibrillate when we consider mass and temperature factors, as instanced by the ease with which the ventricles of the large marine turtles enter into fibrillation (Mills, Garrey).

Garrey has, furthermore, shown that in relation to recovery from fibrillation, when the hearts are subdivided by section, and in this way narrow strips and bridges of tissue block the transmission and spread of the fibrillary state, the behavior of the large marine turtle heart is identical with that of the fibrillating mammalian heart. This is an indication of the identity of the processes involved in the incoördination in both types of tissue.

The difference between auricular and ventricular fibrillation in mammalian hearts is attributed by MacWilliam to differences in the anatomical arrangement of the fasciculi. Garrey found that the tendency to recover was in inverse ratio to the tissue mass, probably because greater mass and thickness of tissue afford the pathways for the development of the fibrillary circuits which sustain the fibrillary state.

Clinical fibrillation. With the above facts before us, we are in a position to interpret, from the experimental data, the conditions for and effects of fibrillation of the heart as it is known clinically; in fact the recognition of auricular fibrillation as a clinical entity is a striking example of the most direct application of the results of animal experiments to the interpretation of a state of disease.

Vulpian in 1874 warned against the therapeutic use of electric currents, either in treatment or resuscitation, for he found that thereby fatal ventricular fibrillation was induced in his animals. This report gives the first suggestion of the possibility of this disturbance in man. It is now well recognized that so-called cardiac death is due to ventricular fibrillation, and that this process may be the terminal event in other clinical conditions (Robinson, 1912; Hoffman, 1911; Hoffman and Selenin, 1912; and R. H. Halsey, 1915). Transitory ventricular

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fibrillation has been demonstrated by Robinson and Bredeck (1917) and recurrent paroxysmal attacks have also been recorded (Kerr and Bender). Levy also claims that the transitory cardiac syncope of chloroform anesthesia is due to a brief stage of ventricular fibrillation which is recovered from. He has seen such ventricular recovery repeatedly in dogs after chloroform fibrillation.

Auricular fibrillation, for sixty years regarded merely as a physiological curiosity, suddenly assumed a position of greatest importance among the clinical cardiac irregularities with the demonstration that it is the cause of the so-called "delirium cordis" or "pulsus irregularis perpetuus." It is now recognized as the commonest of all cardiac irregularities, making up some 50 per cent of the total number (Lewis) and being responsible for from 60 per cent to 70 per cent of all serious cardiac failures (Mackenzie).

Cushny (1899) first suggested that auricular fibrillation might be the cause of ventricular irregularity as seen in man. He published tracings of the form and irregularity of the arterial pulse curves of animals with experimental auricular fibrillation; he compared these with tracings from a human case of delirium cordis, remarking the similarities in form and time relations, and attributing them to a common cause. Fox, and Hewlett, subsequently investigated this case by newer methods and confirmed Cushny's conclusions. Subsequently, Cushny and Edmunds again called attention in two papers (1906–1907) to the probable connection between auricular fibrillation and delirium cordis in man. The irregularity of the ventricles in auricular fibrillation has been noted by many workers in this field, including the first workers, Hoffa and Ludwig, and Vulpian; Phillips (1904) records the same fact from the laboratory of Frédericq.

Frédericq (1904) gave the experimental proof of the relationship between auricular fibrillation and ventricular irregularity; he cut the bundle of His and put an end to the ventricular irregularity, thus showing that the ventricle was responding to impulses from the fibrillating auricles. The phenomena received their explanation when it was shown by Lewis (1912) and independently by Garrey (1912–1914) that any sufficiently narrow bridge of cardiac muscle, whether auricular or ventricular, will suffice to prevent the spread of the fibrillary state, but will allow individual impulses to pass. Garrey showed that any physiological barrier to conduction would act in a similar way, thus tissue compressed by a Gaskell clamp to the stage bordering on partial block, will act like the His bundle or a narrow bridge of tissue and allow

individual impulses to pass irregularly from a fibrillating region to one not fibrillating.

The final step in demonstrating that auricular fibrillation is a clinical entity was taken independently by Rothberger and Winterberg (July, 1909) and by Lewis (November, 1909), who showed that the venous pulse tracings and the electrocardiograms obtained from animals with experimental auricular fibrillation present the same characteristics as those obtained from patients with pulsus irregularis. Lewis added further confirmatory evidence when he recorded six cases of complete irregularity of the heart in horses. One horse was shot through the brain and the heart rapidly exposed. The ventricle was found beating rapidly and irregularly. Lewis says, "At first no intrinsic movement could be seen in the auricle, its walls seemed fixed in diastole, but close inspection revealed the fibrillary movement. Thus came the final ocular proof that experimental fibrillation as we know it occurs as a manifestation of disease." In the clinical cases and in experimental auricular fibrillation the condition is marked by common manifestations. In brief these are: *1*, rapid ventricular tempo with absolute irregularity both as to rhythm and force;* *2*, absolutely irregular arterial pulse; many ventricular beats recorded at the apex or on the electrocardiogram fail to record on the arterial pulse. There is a "pulse deficit" (Draper) which has considerable prognostic significance (Cohn). *3*, The venous pulse is of the ventricular form (Mackenzie, Hering), that is, it lacks the "a" wave which normally heralds auricular systole and precedes ventricular contraction (Frédericq, Hering, Wenckebach). This absence of the "a" wave misled Hering (1909) to believe that the auricle was paralyzed, for which view Mackenzie had also argued before he adopted the equally erroneous idea that he was dealing with a nodal rhythm in these cases. Often the venous pulse records show rapid, irregular deflections of slight amplitude (Wenckebach) which are best seen in the longer periods of diastasis when the ventricle is beating slowly, as after vagus compression or administration of digitalis. Corresponding changes are found in esophageal tracings (Hewlett). *4*, The electrocardiogram shows persistent oscillations of the string which are irregular in tempo and magnitude (Rothberger and Winterberg); like the venous pulse variations they occur throughout the cycles but are best noted in the period of ventricular rest. They do not, however, correspond to the oscilla-

*Rarely the rate may be slow, with irregularity, especially when there is concomitant depressed conductivity.

tions recorded synchronously on the venous pulse (Niles and Wiggers). These oscillations are definitely due to the continuous activity of the auricle (Lewis; Lewis, Drury and Iliescu), but no "P" wave marks a definite auricular systole. The ventricular complex is normal, and this indicates that the ventricular systoles are initiated by impulses of supraventricular origin. The comparisons of venous pulse tracings and electrocardiograms of experimental fibrillation in animals with those of human cases of pulsus irregularis have received ample attention from many American workers (Hewlett, Hirschfelder, Cohn, Robinson, Wiggers, among others). The work of these authors elucidates the clinical state of auricular fibrillation.

AGENCIES INDUCING FIBRILLATION. The misconception is still not uncommonly met with that fibrillation is caused only by agencies which increase the excitability of the cardiac musculature. The view is probably a survival of the old idea of Engelmann (1895) that in fibrillation, the muscle fibers contract independently; that owing to increased excitability, they have become independently rhythmic (Winterberg). This means that multiple ectopic foci of impulse formation must have developed, a concept which by evolution of nomenclature becomes "heterogenesis," so voluminously propounded by Lewis, a concept which became so firmly intrenched in the medical literature that it dies hard even now long after its champion (Lewis) has abandoned it and adopted the conception of circus motions as demonstrated by Garrey and suggested by Mines.

The well-known action of digitalis and the digitalis group of drugs in causing ventricular extra-systoles and auricular fibrillation, is commonly quoted in support of this hyper-excitability idea, as is the similar effect of toxic adenomata of the thyroid gland. The frequency with which extra-systoles are found in association with a weakened myocardium and the transient auricular fibrillation frequently noted, for example, after influenza, diphtheria and pneumonia, as well as the fact that rheumatism precedes some 50 per cent of the cases of auricular fibrillation, and the further fact that such fibrillation may persist for years or may even be permanent,—these are the well-known clinical phenomena which speak directly against the idea that fibrillation of the auricle (or ventricle) is necessarily or even commonly due to any condition of hyper-excitability. The great variety of conditions under which fibrillation is experimentally induced suffices to rule hyper-excitability out of court as a condition essential to fibrillation. MacWilliam definitely stated the fact that fibrillation can, and usually does occur with a generally depressed condition of the cardiac muscle.

Analysis of the data bearing on this question led to the obvious conclusion that fibrillation is a consequence of *relative* differences in the physiological state of different parts of the muscle, independently of the general level of functional capability. The general excitability may be high or it may be low so long as the contiguous regions have different excitabilities and the conduction through them is at different rates or the refractory states do not dissolve synchronously. Such differences establish a physiological condition which disrupts the normal coördinated contractile process and may result in fibrillation.

In enumerating the agencies conducing to fibrillation, it is possible to arrange them in more or less antithetic relation to each other, so far as their effect upon the excitability of cardiac muscle is concerned. It is possible to show that one and the same agency or similarly acting agencies may either produce or stop fibrillation, or that oppositely acting agencies may have similar effects in their relation to fibrillation.

Sudden cooling of the heart was one of the methods used by Kronecker to produce fibrillation, especially local cooling of a twig of the coronary artery; yet MacWilliam and also Porter cooled the heart in order to stop fibrillation. Sudden warming, on the other hand, may cause fibrillation (Kronecker, Schlomovitz) or increase the tendency to fibrillate (MacWilliam), facts which are common laboratory experiences when working with cold-blooded hearts. On the other hand, Barbéra stopped fibrillation by warming to 45°C.

Atropin, which paralyzes the vagus endings, caused fibrillation in the experiments of Cohnheim and Schulthess-Rechberg, also in those of MacWilliam. The latter further states that pilocarpine and physostigmin, which stimulate vagus endings, stop fibrillation, but muscarin, which has a similar pharmacologic action, induces fibrillation. Nicotin conduces to the fibrillary state (Winterberg). While the action of cardiac nerves is to be considered below, it is pertinent at this juncture to state that there is ample evidence to show that vagus stimulation may stop auricular fibrillation. On the other hand, it may induce a state which establishes or favors the abnormal process. Stimulation of the accelerans renders fibrillation, at least of the ventricle, more easy (Hunt, Hering, Garrey), although Winterberg claims that this effect is secondary to circulatory effects through the coronaries. Rothberger and Winterberg (1914) claim that both flutter and fibrillation are apparently shortened in duration by accelerans stimulation, although they report extra-systoles following sympathetic stimulation after barium chlorid as did Levy after chloroform anesthesia.

Potassium chlorid and the potassium containing ash from coffee was used to produce fibrillation (Aubert and Dehn, MacWilliam, Guttmann). It also may render the heart more prone to fibrillate when unusually low rates of stimulation are employed (MacWilliam). It is a depressant of conduction, and as such a 1 per cent solution, used as a perfusate, is one of our surest means of stopping fibrillation (Hering). Calcium chlorid, which has in many respects effects upon the heart muscle quite the opposite of those of potassium chlorid, will also cause fibrillation of the heart when isotonic solutions in small amounts are injected intravenously (Winterberg). Barium chlorid too causes fibrillation; but strontium chlorid, by way of contrast, is one of the salts which stops fibrillation (MacWilliam).

Carbon dioxid, through its depressant action, was one agent used by Porter to stop fibrillation; yet when it is perfused through the coronary arteries, it starts fibrillation; while neutral gases (hydrogen, nitrogen) do not (Magnus).

Digitalis (and strophanthus) increases the excitability and causes fibrillation, but so does bile which is a definite depressant. Chloral hydrate sometimes causes fibrillation, but also will neutralize the action of digitalis and prevent fibrillation (Gley, Barbéra). Other local anesthetics, like chloretone and urethane, are general depressants and applied locally act on heart muscle like the vagus, but they will stop fibrillation (Garrey). Adrenalin is said by MacWilliam (1918) to be most efficient, intraventricularly injected, in stopping fibrillation induced in a variety of ways. Small doses, however, may induce fibrillation, especially when combined with light chloroform anesthesia (Levy).

Camphor, it is claimed (Winterberg), makes the induction of fibrillation more easy, and the recovery from this state more difficult (Klemperer). On the other hand, it is held that camphor stops fibrillation (Seligmann, Gottlieb and Magnus). In these conflicting results, one sees that it depends upon dosage and localization of action whether the drug favors or is inimical to fibrillation.

Interrupted galvanic currents (Hoffa and Ludwig) and faradic shocks (Vulpian) cause fibrillation, but sufficiently rapid and strong alternating currents of high voltage are said to stop fibrillation, especially when coupled with massage (Prevost and Barbéra). The constant current can cause fibrillation (S. Mayer), independently of its heating effects, especially when its anodic and cathodic effects are simultaneously adduced (Garrey). It can be shown that the anode alone will stop fibrillation (Garrey).

A single electrical shock or mechanical stimulus will often cause fibrillation especially when timed to fall immediately after the refractory phase of a preceding contraction (de Boer). This latter condition is far from a necessary one since, given the physiological condition for fibrillation, as the writer will show in a subsequent communication, any single stimulus to a *resting* muscle may cause fibrillation.

De Boer did his experimental work on fibrillation with hearts of frogs after bringing them to an exact and limited stage of impaired metabolic activity by exsanguination. The coronary circulation in its relation to fibrillation of the mammalian heart has had a considerable place in the early literature of this subject and an important bearing on the metabolic state which de Boer emphasizes in this relation. He has therefore devoted much space to this subject in his recent review of fibrillation written for the Ergebnisse der Physiologie (1923). It will satisfy the purpose of this review to state the work briefly. Ventricular fibrillation may follow closure of the coronaries, either by ligation, clamping, or plugging by injections. Kronecker, in his later work, thought that anemia was at the bottom of these results, but that the anemia was secondary to vasomotor nerve injury. Tigerstedt thought these results due to trauma, as did Fenoglio and Drogoul, or trauma coupled with lack of oxygen (Cohnheim and Schulthess-Rechberg). Porter, by plugging the coronaries, showed that this was not true; that *localized* ischemia was the real cause of the fibrillation. A somewhat similar, though less conclusive explanation, might have been drawn from the work of Panum, of Sée, Bochefontaine, and Roussy; and of Martin and Sedgwick. Complete anemia, such as that produced by removal of the heart, does not necessarily cause fibrillation, and Langendorff found that fibrillation often ceased when his perfusate was shut off, thus producing anemia. Hering seems to have been the first one to point out that the essential feature in this coronary fibrillation is the establishment of foci of relatively different physiological or "metabolic" conditions, and Porter saw therein the condition for the development of blocks which divert the course of the normal impulse and thus cause fibrillation.

Auricular fibrillation is essentially a functional condition. There is little reason to lay undue stress on alteration of the muscle to account for the abnormal rhythm. Search for pathological lesions in the sinus node or elsewhere has failed to yield satisfying findings (Cohn). It is recognized that the incidence of the clinical condition is most

frequent at the age of about 50 years, and that 50 per cent of cases have a history of rheumatism, and further, that anatomically there may be subacute or chronic inflammatory changes leading to fibrosis. Similar findings are, however, more frequently present when there has been no fibrillation, and fibrillation may occur spontaneously in the young with apparently healthy hearts (Gossage and Hicks). The ease of its experimental production in healthy hearts of animals by chemical, electrical and mechanical means speaks almost positively for the purely functional nature of the process. Any agencies which can bring about local differences in the physiological properties of the musculature may induce fibrillation, e.g., inhalation of hydrogen sulphid (Robinson).

Among the general physiological changes which have been regarded as causes of fibrillation are slowing of conduction, heightening of excitability, shortening of the refractory period, and establishment of intramuscular blocks or localized obstacles to the passage of the contraction wave. It is obvious that inflammatory or toxic influences may conduce to such changes, for example, localized interference with the blood supply, either through emboli or thrombi, or by arteriosclerotic changes; but equally, we recognize that the same effects may be brought about by chemical means or by nervous influences, especially when accompanied by acceleration in heart rate such as one notes after emotional crises (Kuré, Mackenzie), in exophthalmic goiter, or after severe work.

THE NATURE AND THEORIES OF FIBRILLATION. Fibrillation, such as that described above, was referred to by Heidenhain as a "tumultuous tetanus." Schleski speaks of it as a "tetanus with intermission." Both Goltz and Cyon refer to it as a tetanus, although Cyon also refers to fibrillation in the frog's ventricle as a peristaltic contraction. The impossibility of a heart tetanus disposed of that conception.

Aubert and Dehn (1874) believed that potassium (salts) caused fibrillation by injury to a "coördination center"—a nervous structure in the heart. Kronecker and Schmey, by puncture of the ventricular septum at its upper third, produced fibrillation. Kronecker argued that the operation destroyed the coördinating center, which he also thought was a nerve structure. Of course, a similar center would have to be postulated for the auricles, since they can beat or fibrillate independently without involving the ventricles. The idea of such a center was untenable after MacWilliam demonstrated frequent spontaneous recovery from such fibrillation, even in the case of the dog's

ventricle. He showed that the apex could fibrillate and recover repeatedly, although it was supposed to possess no nerves. Porter successfully stopped fibrillation of any isolated piece of the heart and showed that it could beat regularly in spite of its separation from any hypothetical coördinating center. Garrey obtained similar results. Kronecker subsequently changed his views, and proposed the explanation that his puncture interfered, by stimulation of vasomotor nerves, with the blood supply to the heart and thus produced fibrillation in the same way that tying a coronary artery does. This conclusion, like his earlier one, was completely disproved by the work of Langendorff.

Cohnheim explained the delay in the onset of fibrillation following coronary ligation, as due to the slow development of a "metabolic poison" which acted as an unidentified stimulus. Just how such a stimulus could cause fibrillation was not made clear.

MacWilliam was the first to make a real attempt to analyze fibrillation, and present a plausible explanation of the abnormal type of irregular contraction. He showed that it could take place with either increased or decreased excitability. He brought out clearly that usually conduction is decreased, that the impulse progresses at different rates and "reaches adjacent muscle bundles at different points of time, and, since these are connected with one another by anastomosing branches, the contraction would naturally be propagated from one contracting fiber to another over which the contraction wave had already passed." "There would evidently be a more or less rapid series of contractions in each muscle bundle in consequence of the successive contraction waves reaching that bundle from different directions along its anastomosis with other bundles."

MacWilliam did not show that this was a continuous process and not a transitory one. He elsewhere falls back on the assumption of ectopic foci of impulse initiation and thus missed the essential features which convey the idea of "circus contractions," as developed by Garrey, an idea which, however, MacWilliam espoused over thirty years after the appearance of his original communication. Porter, in 1894, added materially to our conception of fibrillation by proposing the "block hypothesis;" he postulated the development of intramuscular blocks which served to deflect the course of the impulse and to conduct it into abnormal and devious paths, so that it may be conceived as shuttling about or weaving its way through the mass of cardiac tissue. The suggestions of these two workers were fundamental in that they

laid the basis for the subsequent theory that fibrillation is a circus motion.

Struck by the fact that different parts of the fibrillating heart chamber are not in the same phase of contraction, Engelmann (1895) proposed that, in fibrillary contraction, the different heart fibers become independently rhythmic, and that each is a focus of impulse formation. When we conceive that these independent, multiple ectopic foci have inherently different rhythms, and that in addition, each may be beating at its maximal rate (F. B. Hofman, 1905), we have an idea of the degree of incoördination which is believed to prevail in fibrillation. This theory was especially elaborated by Winterberg (1906), and was adopted by many prominent workers, including Rothberger and Winterberg, Hering, Rihl, Haberland, and Lewis; and for this reason it has dominated the literature for years. Lewis developed a special modification under the caption "heterogenesis," which he recently (1920) repudiated to espouse the idea of circus motions proposed by Garrey (1912, 1914), Mines (1913), and de Boer (1919–1920). Rothberger and Winterberg also have changed their views, as will appear below.

The theory of Engelmann has been variously known as that of multiple ectopic beats, multiple extra-systoles, heterotopic impulses, polytopic impulse formation, myoerethetic irregularity, functional fragmentation, and heterogenesis. It is based on the fundamentally erroneous idea that fibrillation depends on increased excitability, whereas we know that reduced excitability and conductivity are the rule. Erlanger and Blackman showed that the auricular appendices of the dog are not independently rhythmic in blood. While they can beat in certain abnormal solutions (Garrey), they cannot be conceived as assuming, in the intact organism, any such degree of rhythmicity, if any at all, as that which the theory demands. Different parts of the fibrillating auricles (or ventricles) do not contract as the result of independent impulse formation. This was shown by Garrey who cut away *small* pieces of the fibrillating muscle, or functionally severed them from the main fibrillating mass by clamping. Each severed piece in turn ceased to fibrillate and came to rest, though remaining excitable and contractile. When enough of these pieces had been successively cut away to make a sufficient reduction in the mass of the remaining tissue, it too ceased to fibrillate and again contracted coördinately in response to the normal rhythmic impulses. This experiment was crucial, it showed that the individual fibers are not independently

rhythmic, that their fibrillation depended on their relation to other fibrillating tissue, and further that if the size of the tissue mass is small, the mechanism for sustaining the fibrillary process (believed to be a continuous circulation of the impulse) cannot be effective.

Rothberger and Winterberg have more recently (1914) rejected the view of polytopic impulse formation and dissociated contractions. They have substituted another hypothesis according to which fibrillation is merely a tachysystole, that is, a tachycardia of exceptionally rapid, coördinated contractions. It is based on a series of records taken by the Garten-Clement method; the electrocardiographic tracings show that the sharply localized disturbances cause electrical oscillations of more or less regularity, which are interpreted to mean rhythmic and coördinated contractions in the fibrillating auricles, and at the astonishing rate of 3000 to 3500 per minute. If the inciting faradic stimulation is stopped, the frequency drops gradually, the fine waves give way to coarse ones, auricular flutter sets in, the electrical oscillations drop to 500 per minute, and, after pause, the normal rhythm sets in. If the vagus is stimulated just before the expected change to a normal rhythm, the oscillatory rate again jumps to 3000 or more, and the fine oscillations of fibrillation are brought back. The authors claim that successive oscillations have the same general form, and that the rhythm corresponds to that of the mechanical contraction recorded from a distant point, therefore they maintain that, at least transitorily, the two points have a common abnormally located pacemaker as the hypothesis demands. These authors show that such rapid contractions require that there shall be a marked shortening of the refractory period. In another connection, Lewis, Drury and Bulger agree that the refractory phase is materially shortened at these rapid rates, and have shown that under the influence of vagus stimulation, it may be shortened to one-fifth or one-sixth its regular duration. Only as a result of vagus influence does this extreme change take place. Ventricular fibrillation (cat) shows frequencies between 400 and 800 (Rothberger and Winterberg). It would appear then that very high frequencies are not essential to fibrillation, at least of the ventricle, and that vagus influences are likewise not essential, for Garrey found that the vagus influenced only 16 per cent of his series of fibrillating dogs' ventricles, and Rothberger and Winterberg report no effects observable in their experiments.

In the presentation of the above hypothesis, its authors state the belief that the difference between auricular flutter, with a frequency

around 500, and fibrillation with 3000 to 3500, is solely one of rate, and that experimentally and clinically either form of abnormality can slip over into the other type. MacWilliam was the first to show the experimental relationship between flutter and fibrillation of the auricles, and Hewlett (1907) noted the same fact. In referring to his experiments in the same field, Robinson (1913) says, "That the abnormal activity in man passes from a state of flutter to that of fibrillation in a similar manner may be taken as evidence for the belief that auricular fibrillation and auricular flutter in man are closely allied cardiac disorders" (*cf.* Ritchie, *infra*).

The extreme frequency of auricular systoles is held to account for the mechanical weakness of the contractions and presumably this bespeaks a weakly conducted impulse, so that there is atrio-ventricular block. The block may be constant in ratio, with a constant ventricular rate slower than that of the auricle, or it may be a changing ratio as from 2:1 to 3:1 or 4:1, thus leading to irregularity of the ventricles.

Recently Rothberger (1922) reaffirms that the "tachysystole, in and of itself, is sufficient for a complete explanation of all the phenomena, and therefore may be designated as a cause of flutter and of fibrillation," although he (and Winterberg) agrees that the theory of circus motions may be correlated in such a way as to offer a better explanation of the phenomena, e.g., circus motions, producing a tachysystole.

When we analyze the theory of Rothberger and Winterberg on the basis of other experimental evidence, we find much that does not harmonize with the idea that fibrillation is a simple tachysystole. With a rate of 3000 per minute only a fiftieth of a second elapses between impulses. A slow conductivity favors fibrillation and one may assume a propagation rate of 500 millimeters per second in the auricles (Lewis). Under these conditions wave fronts would be only 1 cm. apart and there would be many propagation waves all travelling in the same direction between the points of initiation and the most distant points. It is hardly conceivable that such a condition could involve an entire atrium and continue for years as it is known to do.

Kisch, after studying the electric changes in neighboring points on the fibrillating auricle, concluded that the contractions were not isorhythmic, although Rothberger disputes the conclusion. Wiggers, in his study of the mechanical changes of fractionate contractions of the fibrillating auricles, showed conclusively that the impulse pro-

gresses in a zigzag path, that the tissue between the two points of attachment to recording devices may contract as a whole or in part, that one part may be relaxing when another is contracting. These and other evidences, we feel, indicate complete incoördination.

MacWilliam has shown that a single beat may fibrillate, and in a forthcoming publication Garrey will show that such fibrillary beats are initiated in the normal way from the normal pace maker, that the impulse shuttles back and forth through the auricular muscle, producing rapid oscillations which may stop before the next impulse arises, so that the normal rhythm is not disturbed in the least; there is no "post-undulatory pause" in such cases. The normal impulse from the sinus region, or any other impulse, no matter where it originates, may cause fibrillation and the wildest incoördination of shuttling impulses if only the musculature is in what may provisionally be called the "fibrillary state." The theory of tachysystole is not consistent with these facts.

The low ebb of excitability and conductivity which favors fibrillation, e.g., localized cooling or exsanguination with its consequent "bad metabolic condition" of which de Boer speaks, are not consistent with the development of a hyper-rhythmic ectopic focus. Vagus stimulation has been shown to change flutter into fibrillation, but it depresses all properties of cardiac muscle. Is it not then far-fetched to attribute to vagus stimulation the development of hyper-rhythmicity in any ectopic focus of impulse formation, a hyper-rhythmicity such as would be necessary to account for a change from 500 to 3500 impulses per minute? Vagus action can be explained otherwise.

Excessively short refractory periods are essential to the validity of Rothberger's view, but a refractory phase sufficiently short for 3500 contractions per minute can develop only in the auricles. It can develop only under the influence of strong vagus stimulation (Lewis) such as one resorts to in laboratory experiments. No such excessive vagal effects can be dreamed to be effective through the years of chronic auricular fibrillation seen clinically. Furthermore, fibrillation persists after atropin and vagus paralysis; in fact, atropinization was one of the first methods used by MacWilliam to produce fibrillation. Any ventricle can fibrillate, although here vagal effects are against the rule, and the refractory period, even at its shortest, is longer than that of the auricle normally. Accelerans stimulation can cause ventricular fibrillation, but this result cannot be attributed to shortening of the refractory phase. Fibrillation in any part of the heart of cold-blooded

animals involves muscle whose refractory period certainly is not short and has not been shortened. In fact in the turtles' auricles, oscillations of the string of the galvanometer are at a rate of 20 to 30 per second, 1200 to 1800 per minute. Coördinate contractions at such rates are impossible in these hearts because the refractory period is far too long. The oscillatory rates are to be explained by the shuttling of a single impulse in the vicinity of the electrodes. Shortening the refractory period indeed favors the finer types of fibrillation, renders the onset more easy, conduces to its persistence, but it is not a necessary condition for fibrillation as the theory of tachysystole demands. De Boer shows actual lengthening of the refractory period in the fibrillating, exsanguinated heart of the frog.

Garrey's work has developed evidence along different lines, which directly contradict Rothberger and Winterberg's fundamental conception of fibrillation as a coördinate tachysystole. Garrey showed that narrow strips of auricular (or ventricular) muscle cannot be made to respond to any such rapid rate of stimulation as the theory demands, and will not fibrillate, although larger tissue masses readily do so. Mere inspection frequently suffices to show that impulses do not pass over different regions of fibrillating muscle at the same conduction rate, and this fact alone spells incoördination and prevents true tachysystole. When the margins of a strip are functionally separated from the rest of the auricle, either by a clamp or cautery, in such a way as to leave both its ends functionally open to stimulation from the fibrillating muscle, it is found that impulses course over it sometimes in one direction, sometimes in the opposite direction. This could not happen if the stimuli arose in a single pacemaker and progressed coördinately from one point as demanded by the theory. The fibrillary process will not extend into a narrow strip cut from a fibrillating auricle or ventricle and the fibrillary process will not extend across a narrow bridge to non-fibrillating muscle, although there is no inability to conduct impulses or to respond to simple stimuli as readily as any other part of the musculature. From these results it is justifiable to conclude that no part of the heart can respond with coördinate beats to the excessive rates demanded by the hypothesis under consideration. This conclusion applies no matter what conditions prevail.

When one cuts or clamps a finely fibrillating heart chamber so as to divide it into two or four equal parts, each continues to fibrillate although obviously only one of these pieces can contain the original, hypothetical, tachysystolic pacemaker,—at least three of the pieces

should have stopped fibrillating instantly. We are forced by these results, either to fall back on the idea of polytopic impulse formation, which we have seen is equally an untenable explanation of fibrillation, or to adopt a new and more plausible explanation. Rothberger (1922) concedes that the theory of circus motions probably offers an explanation of these experimental results on which he based his idea that fibrillation is a tachysystole.

THE THEORY OF CIRCUS CONTRACTIONS. The results of section of fibrillating hearts, as outlined above (Garrey), the relations of tissue mass and form to fibrillation, and the interrelation of parts of a fibrillating mass, demanded an explanation which the previously current theories did not provide. Garrey conceived the explanation that an impulse can travel in a ring circuit, return upon its old path after the refractory period has passed off, and thus continue to circulate. Experimental proof of this came to hand prior to reports made in 1912 and 1913, and a more complete report was published in 1914. Garrey stated his theory in the following words:

> Impulses can spread in any and all directions, their progress being limited only by the preexistence or development of localized blocks within the tissue mass. Such blocks divert the impulse into other and more circuitous paths, and the area so blocked off can participate in contraction only when an impulse which has passed the other parts of the ventricle approaches it from another direction; this area thus in turn becomes a center from which the progress of contraction is continued, to be in turn diverted by other blocks. The existence of such blocks, and especially of blocks of transitory character and shifting location, has been noted in the experiments detailed above. These conditions make possible the propagation of the contraction wave in a series of ring-like circuits of shifting location and multiple complexity. It is in these *circus contractions*, determined by the presence of blocks, that we see the essential phenomena of fibrillation.

It will be noted that Porter's block hypothesis shares in this explanation; so does MacWilliam's view. Garrey further stated: "It is conceivable that the establishment of *relative* differences in the excitability and conductivity in different parts of the musculature without the condition of absolute block, might result in the same phenomena of circus motions and fibrillation."

Quite independently, Mines (1913) came to similar conclusions, reported "reciprocating rhythm," and described the mechanism by which an impulse could continue to circulate in a ring of heart muscle. Normally the impulse courses so rapidly and the refractory period lasts so long that only one wave can occupy the heart at one time.

With a slow conduction, a short refractory period and a high frequency, more than one wave can be present and the net-like structure makes closed circuits possible. Travelling in these closed circuits, the wave front does not complete the circuit until the refractory period has passed off, hence it meets no obstacle to progress and can continue to circulate. At the time of the publication of this fundamental work, Mines had done no experimental work on fibrillation, but he clearly conceived and suggested that this mechanism might offer an explanation, at least of frequent extrasystoles, and possibly of fibrillation. The independent work of Garrey and of Mines established an entirely new principle in cardiac physiology and functional pathology, a principle which has been accepted quite generally, claiming among its adherents and proponents the chief workers in this field; MacWilliam, Robinson, Cohn, de Boer, Levy, Lewis and his laboratory workers, and with reservations, Rothberger.

De Boer, in 1920, on the basis of his own earlier work, proposed the same explanation of the nature of fibrillation as it appears in the ventricles of the frog. He noted that a single stimulus, applied some time after the refractory period was over, caused an extrasystole, whereas a stimulus applied immediately after the refractory phase, caused a continuous circulation. In the latter case, as Mines had previously suggested, the weak contraction was followed by a short refractory phase and permitted the circulation. De Boer, like Garrey, presupposes blocks. He assumes that these blocks break up the musculature into a number of functionally distinct masses. The impulse involves these units successively so that each contracts as a whole, and the wave thus proceeds by jerks due to these partial systoles. De Boer's tracings bear out his statement that, in the frog's ventricle at least, each circuit involves the entire ventricle. This might have been expected in so small a heart as that of the frog. Single circuits are common phenomena, especially expressed in the "Wühlen und Wogen" of cold-blooded hearts, even of large size. They are not by any means the exclusive expression of circus contractions, as we shall demonstrate. Given the fundamental conception of circus motions, with a comprehension of the significance of blocks, varying in position and degree, given an appreciation of the importance of relative differences in excitability and conductivity and of changing refractory periods, one is in a position to form some idea of the variety of ways in which circus motions can express themselves, and the possible degrees of complexity of these expressions. We see brief shuttling impulses within the time of

a single normal cycle. We see a simple, single circuit causing an extrasystole superimposed on and continuous with the preceding normal beat. We see a simple circuit involving the whole structure successively and continuously in its circulating progress. We see impulses sharply localized and limited to a circumscribed path within a part of musculature repeating their single course in even tempo, thus causing a regular coördinate flutter in the rest of the auricle, or we see them cause wild, localized fibrillation like that produced in the region of stimulating electrodes. The latter condition forces irregular, but still fluttering, contractions of the rest of the auricle. Finally, we see the wildest turmoil of fibrillation involving the whole auricle in a process which is anything but a simple circuit. In this last mentioned process, the impulse is diverted into different paths, weaving and inter-weaving through the tissue mass, crossing and recrossing old paths again to course over them or to stop short as it impinges on some barrier of refractory tissue. In these different variations of circus motions, we have sufficient basis for the variety of arrhythmias which we designate as simple or frequent extrasystoles, flutter, impure flutter, coarse fibrillation, paroxysmal tachycardia and fine fibrillation. No matter what condition of simplicity or complexity develops, it is still covered by the terms of the original theory of circus motions as formulated by Garrey. The simple circuit of de Boer was well known to Garrey at the time he formulated his theory of circus motions or he would never have had the temerity to propose it. That fibrillation develops spontaneously was an equally familiar fact, and that fibrillation could be started by a single stimulus was also known from both the literature and personal experience.

Many investigators do not know that a single stimulus, if properly timed, can stop any *simple* circuit. This is a fact which speaks emphatically against the conception that all fibrillation consists in a single circuit, for all fibrillation cannot be stopped in this way. The idea of "multiple circuits" as stated by Garrey in the above quotation has been misinterpreted or misrepresented; it does not mean, and never was intended to mean, that multiple circuits were fixed in their isolated paths and independent of each other, that there was no fibrillation except when there were multiple circuits, but it *was* intended to convey the idea that the impulse looped back upon its old path, completing a circuit, now here, now there. Such reëntrant paths can exist side by side transiently. In this way, and only in this way, did multiple circuits enter into the theory. If the only true fibrillation is the

fine type of fibrillation, as has been maintained by MacWilliam, Rothberger Lewis and Wiggers, then true fibrillation is never made up of a simple circuit but ever of shuttling impulses which make many and various circuits as described above.

The simple circuit: This form of continuous contraction was first recognized by the present writer in 1911. In a turtle's excised heart, the auricles were seen to beat in perfect alternation, each at the rate of 105 per minute. In attempting to cut the circuit, the initial mechanical touch of the scissors stopped the abnormal process and the auricles beat together normally at the rate of 36 per minute. The phenomenon has repeatedly been seen since then. Simple circuits can be studied to advantage in the heart of large Selachians where the wave may travel so slowly that it can easily be followed ocularly (10 to 12 cm. per second). The following experimental results will give an idea of possibilities of the simple circuit. In the first place, the normal rate of 10 per minute was superseded after generalized faradization, by a contractile impulse which was seen to circulate in a peristaltic sort of undulation completely around the auricle from side to side, completing a circumferential circuit of 12 cm., 60 times a minute. The ventricle followed only half the circuits and beat 30 times per minute. Secondly, after a period of rest, faradization of the auricular surface, roughly along the median sagittal plane, started a circuit which was confined to a ring in this median longitudinal line, the circumference measured approximately 6 cm. and the rate was 120 per minute. The auricular appendices both contracted simultaneously and quite coördinately. The ventricle followed every fourth circuit about the auricle. In the third instance, local faradization, confined to one spot on the auricle, started a circuit which coursed irregularly about that spot. The diameter of this intramuscular circuit was indefinite, something like 1 cm. or more. The entire remainder of the auricle responded to each circuit with a coördinate contraction repeated regularly 159 times a minute. This is entirely comparable to the type of flutter mentioned by MacWilliam as due to impulses arising at the point of auricular faradization and sustained from that point, except that they recur more regularly and are sustained long after the stimulation is stopped.

These experiments, while new, have been submitted here to show that simple circuits may involve, not only the entire musculature, as de Boer claims, but also may exist *within* the substance of the auricle and may persist in a definite path which is blocked off from the ex-

traneous tissue so that a coördinate contraction of the outlying tissue follows each circuit only. The phenomena are so easily followed in Selachian hearts, and the results are so unequivocal, that they are presented here in rebuttal of the criticism that simple waves cannot be sustained within the muscular mass. It should be emphasized at this point, that any of the circuits described above could be stopped at will by a single stimulus properly timed; Mines had noticed this result in connection with his reciprocating ring contractions.

Flutter: During the years 1920 et seq., there has appeared a series of papers from the laboratory of Lewis furnishing data for a complete confirmation of the theory of circus motions and correlating laboratory and clinical findings on the basis of this theory. This was first accomplished in a study of "flutter." This condition was first recognized by Herz and Goodhart (1909). It was studied in detail by Jolly and Ritchie as a clinical entity in which the auricle beats rapidly, 240 to 350 per minute, the ventricles following at half or quarter this rate. Lewis (1920) noted a remarkable regularity in the auricular sequence; the intervals between beats did not vary more than (0.01) one hundredth of a second; thus regularity, rather than rate (Rothberger and Winterberg), marks this affection as a thing *sui generis*. Lewis proposes to limit the use of the term "flutter" to the absolutely regular type. This distinction between "pure" and "impure" flutter is decidedly too finely drawn and artificial in view of the similar mechanism underlying both conditions. They are essentially the same thing and should be treated as such, whether of the pure or impure type. Lewis, Feil and Stroud, in two papers, showed that it was possible to produce experimentally pure flutter as well as the impure type by faradization and by rhythmic stimulation of the dog's auricles. The rate of flutter bore no relation to the rate of stimulation; it outlasted the stimulation and could be perpetuated from a locus outside the region stimulated. They proved, by a study of the progress of the electrical disturbance which accompanied the contraction, that a complete ring-like circuit was traversed with each contraction of the auricle. It was found that the wave of contraction circled in one direction in the natural ring of muscle formed by the orifices of the two venae cavae, and that there was a revolution of the electrical axis through 360 degrees with each cycle. Clinically a similar change in the electrical axis was found by electrographic studies with leads taken from the chest over the auricle. On the whole, the flutter rates in experimental dogs were between 345 and 580 per minute, about 50 per cent higher than in human cases.

Lewis thinks that differences in the size of the hearts, and therefore in the length of the ring-like circumferences, is sufficient to explain the differences in rate. Correspondingly, differences in rate from 240 to 350 per minute in the human being can be accounted for by differences in diameter of the natural orifices. At 240 per minute each cycle lasts 0.25 second. Lewis shows that with a transmission rate of 500 millimeters per second, the circumference of the circuit would be 125 millimeters with a diameter of 4 cm. At 350 per minute, the diameter of the rings would be 2.7 cm.

Erlanger has personally communicated to the writer an unpublished observation bearing on this question. He has induced flutter, and subsequently stopped the process, by tightening a clamp, one blade of which was introduced into the heart through the superior vena cava, and the other on its external surface and so disposed that the ring of muscle about the large veins was blocked by the pressure. Garrey finds that he can suppress flutter in many cases by a single stimulus properly applied. We here have two observations which make it doubly certain that experimental flutter is due to a simple circus motion, and that in certain cases (regular flutter) the circuit traversed is about the mouth of the large veins.

In this connection, the reader's attention is directed to the fact that each circuit is accompanied by a single coördinate contraction of the auricle, and that the rate is determined by the diameter of the circuit traversed. This can only be interpreted to mean that one impulse, and only one, is given off from the circumference of the circular path with each circuit. This accounts for the regularity of the flutter in these cases. With many centrifugal waves leaving the ring path in each circuit, as postulated by Lewis, the rate would usually be irregular, and the contractions certainly would be more frequent than one for each revolution of the electrical axis. The refractory period, as well as the diameter of the circuits, would enter into a determination of the rate and regularity. It is possible with several successive centrifugal waves, given off in each circuit, to have very irregular flutter of the outlying auricular tissue.

Natural rings of tissue are not essential for the maintenance of the circus contractions. Garrey has described how this can take place in the Selachian heart (*vide supra*). It was shown in the sustained impure flutter, first described by MacWilliam. The irregular impulses can go out from the region stimulated, at least in the early stages of the stimulation. Garrey repeatedly saw sustained flutter arising from

impulses originating about the electrodes and continuing to arise there after stimulation was stopped. This was proven by clamping off the region and thus stopping the flutter, although the incoördination continued at this point. Such an impure flutter is really the result of a localized fibrillation. The rest of the auricle responds irregularly and rapidly to stimuli which are derived from irregularly shuttling impulses, circulating within the localized region of fibrillation.

Localized fibrillation is no novelty in the literature. It was described by Hoffa and Ludwig, MacWilliam, Porter, Garrey (1908), Lewis. Roth described left-sided auricular flutter, and elsewhere we have a description of one whole ventricle fibrillating while the other responded coördinately (von Bezold). The phenomena described by Rothberger and Winterberg (1910) belong in this class of partial fibrillation, and it is almost certainly a modification of this process which Robinson (1913) described as a combination of tachycardia and fibrillation; although Rothberger and Winterberg did not accede to this interpretation. When the fibrillating foci in these cases are clamped off, the fibrillation ceases, but this is not the case with generalized fibrillation. In these foci, after local stimulation, electrical oscillations have been recorded by Lewis and Cotton at the rate of 1500 to 2400 per minute. The shuttling of a single impulse will account for this high rate, and it is very questionable whether the oscillations represent the rate of true circuits. The outlying tissue shows oscillation rates of 350 to 500 per minute. To account for the high rates recorded near the stimulating electrodes, Lewis and Cotton concluded that local vagus effects and consequent shortened refractory period came into play. It may be mentioned, however, that such local effects can still be produced by local stimulation after atropinizing the auricles (Winterberg to the contrary notwithstanding), and similar effects can be produced by local stimulation of the ventricle although the responses are not so rapid.

General fibrillation: In the sense outlined above, flutter and fibrillation may exist side by side in the same atrium. Whether one condition or the other shall dominate the picture will depend upon the relative masses of atrial tissue involved in the two processes respectively, and to some extent on their relative location. Extend the area of localized fibrillation progressively, and the flutter passes into general fibrillation. This happens with prolonged, strong, or repeated local stimulation. Ritchie (1910) states that, "there may be many transitions from flutter to fibrillation and vice versa, or again, between

flutter, fibrillation and normal rhythm. During these transition stages it may be difficult to determine whether the auricles are in flutter or in fibrillation." Lewis has emphasized the transition stages, but these transitions are not always seen; Garrey finds that general fibrillation involving the whole auricular tissue may be precipitated instantaneously, for example; the impulse may shuttle irregularly through the tissue producing a single fibrillary beat. A succession of such fibrillary beats may occur without disturbance of rhythm. Again, a single normal impulse, or a single stimulus, may precipitate lasting fibrillation involving the whole musculature. How the impulse may shuttle and interweave and continue to circulate has been described above, but Lewis has recently presented another conception of the process; he believes that as in flutter, there is a simple, but irregular "mother wave" circulating continuously in a sinuous path around an ill-defined ring throwing off into the outlying muscle, with every cycle, a number of centrifugal waves which cause and perpetuate its flickering fibrillation. The evidence for such a circulating mother wave is not as convincing as in flutter, and there is much to be said against the view. A single circuit of this sort should be stopped by a single stimulus timed to fall into the open excitable part of the ring, but a single stimulus rarely stops general fibrillation. It should be easy to break such a simple ring circuit and thus stop all fibrillation by cutting or clamping the tissue into two or four masses. Instead of this result, we get two or four separate fibrillating masses. On Lewis' view it is necessary to assume the development of a single new subordinate "daughter ring" in each piece. While this is possible, it is wholly improbable. Such an assumption is gratuitous and is not necessary on Garrey's theory.

Lewis and those working with him have furnished new proof for many details of the mechanism by which circus motions cause flutter and fibrillation. It seems probable that other irregularities, such as extrasystoles, single and grouped, also coupled and alternating beats can be due to circus contractions. The evidence of circus motions is proven for clinical cases as well as for experimental animals. It has been shown that the circuits in auricular flutter are maintained at a slow conduction rate, which is as low as 500 mm. per second, or about one-half normal. A shortened refractory period plays a similar rôle. In auricular fibrillation, a slow wave front is maintained by the presence of multiple blocks which cause a sinuous path or actual shuttling of the impulse. The refractory period may be shortened to one-fifth

or one-sixth the normal, although vagus action is a necessary accompaniment of such high-grade shortening. This change makes rapid reëxcitation possible and the formation of circuits having diameters of but a few millimeters. Since the refractory phase does not occur simultaneously in all fibers, the circuits must be formed in different and shifting locations. All such circuits have an open, excitable stretch which makes a continuous circuit possible, and the object of therapy is to close the ring, to render the entire circuit, if only momentarily, inexcitable. One method of accomplishing this would be to lengthen the refractory period and this at present seems to be accomplished best by the administration of quinidine (Frey, Hewlett and Sweeny; Levy, R. L.; Jackson; Cohn and Levy; Drury and Iliescu; Lewis, et al.). Hubert Mann has suggested that in recovery, when the circuits have been sufficiently slowed by reducing the conductivity (e.g., by quinidine) a normal impulse from the sinus node may be interjected into the circuit and complete its refractory condition. This conception is fundamentally sound and in every way comparable to the mechanism mentioned above by which a single stimulus could stop any simple circuit if properly timed. Further consideration of the recovery mechanism is beyond the scope of this review.

THE VAGUS NERVE AND FIBRILLATION. Reports of the action of the vagi upon fibrillation are often contradictory and seemingly paradoxical, but the apparent conflicts are usually explicable. MacWilliam states that by vagus stimulation frequently the contractile movements of the auricle are suspended, only to reappear when the inhibitory influence has passed off; also he says: "Often movement is entirely arrested and does not recur; the normal action of the auricles goes on after the period of inhibition has passed." The effect was much more certain in the stage that he called flutter or pseudo-fibrillation. These findings are entirely verified by Knoll, Fischel, Phillips, Garrey, Robinson, Lewis et al. Garrey finds that the contrary result reported by others may sometimes be explained by the fact that only one vagus was stimulated; it is desirable to produce vagus depression equally and maximally throughout the whole atrium by stimulating both vagi. This often aids materially in completely arresting fibrillation. Rothberger and Winterberg denied complete suppression, stating that the vagi merely suppressed the inotropic properties, that the fibrillary process continued and again became evident as soon as vagal effects wore off sufficiently to permit contractions. MacWilliam showed that this is sometimes true. Garrey has recently determined that

the renewal of fibrillation in these cases is due to the fact that before the vagus effects upon block and shortened refractory period are recovered from, a *new impulse* from the sinus region may be interjected. In these cases the heart is absolutely quiet, but it is the physiological state (due to the vagal stimulation itself) which makes fibrillation possible should a single impulse start its zigzag path through the auricles. Obviously if the vagus effects on the pacemaker outlast the other effects or if new impulse formation is prevented, there will be no return to the condition of fibrillation.

That the vagus plays a dual rôle in its relation to auricular fibrillation was indicated by the work of MacWilliam, of Knoll, and of Cushny and Edmunds. MacWilliam noted that the vagus did not always suppress fibrillation and that in individual hearts, perfused with ox blood, stimulation of the vagi might have quite the opposite result and cause the hearts to fibrillate. When the auricles are being directly faradized, fibrillation is more apt to result if the vagus is simultaneously stimulated (Winterberg, 1907, 1908; Hewlett, 1908; Robinson, 1913). Hewlett (and Dozier) (1908) have shown that the coarser fibrillation of the auricles was converted by vagal stimulation into fine, hardly visible fibrillation; that the auricles dilated; and that the auricular waves disappeared from the venous pulse (Rihl). Winterberg (1907) noted the change to fine fibrillar twitchings, as did Lewis and Schleiter. Winterberg showed that these effects outlasted the direct stimulation, while without vagus stimulation the heart assumed its normal rhythm as soon as the direct faradization stopped. Winterberg further found that atropin and other drugs which interfere with vagus stimulation, prevented the vagal production of fine fibrillation, and that physostigmin, pilocarpin, muscarin and similar vagal stimulants conduce to this type of fibrillation and render it more easy; that after their administration, the strength and duration of direct faradization necessary to induce fibrillation is materially decreased, and that the after effects are more persistent. After physostigmin, Winterberg found that vagus stimulation alone might bring on auricular fibrillation, and Robinson finds that in some hearts, vagus stimulation alone is capable of initiating fibrillation. This all agrees with Garrey's more recent experience, that the vagi merely establish a condition which makes circuits easy should an impulse be thrown into this tissue. Robinson finds that vagus stimulation may establish or "fix" fibrillation in otherwise refractory hearts. The well-known tendency of digitalized hearts (auricles) to fibrillate (Mackenzie) and the easy transformation of

flutter into fibrillation by digitalis or strophanthus is attributable to vagus effects, probably by establishing blocks. Rothberger and Winterberg (1910) described fibrillation due to reflex dyspneic vagus stimulation, and suggest that this is one possible explanation for the common incidence of fibrillation in the hyperexcitable.

Important as the vagus effects undoubtedly are for the development of auricular fibrillation we must be guarded against losing sight of other factors. Atropinized auricles *can* fibrillate; so can the ventricle where the vagus plays a minor rôle. Many conditions conducing to fibrillation have been enumerated in a previous section of this review, conditions which are wholly unrelated to vagus action. They may of course be conceived as instituting conditions similar to those which the vagus can produce. In this connection, Cushny, and also Garrey, have recorded instances in which *section* of the vagi caused fibrillation. In explanation of this result, de Boer thinks the increased rate caused normal beats to enter a damaged heart muscle just at the close of a refractory period, a relationship which he shows may produce fibrillation.

Kronecker and Spallitta (1905) showed that vagus stimulation slowed the ventricles during auricular fibrillation, and records of similar changes were made electrographically (1910) by Rothberger and Winterberg. Robinson (1913) cut the vagi of dogs with auricular fibrillation and noted the increase in ventricular rate with the removal of the tonic vagus effects on the His bundle. Robinson, and Robinson and Draper have shown delayed auriculo-ventricular conduction and block produced by vagus stimulation, and noted that the auricular deflections on the electrocardiogram continued. These a-v conduction effects have been demonstrated on human cases of auricular fibrillation (Rihl, Robinson). Kilgore (1919) reported cases of auricular fibrillation with periodic tendencies to acceleration and retardation of ventricular rate; the periods corresponded to respiratory movements. He attributes the effect to respiratory neurogenic (vagus) fluctuations in bundle conductivity, but notes that periodic changes in auricular fibrillary contractions may be a factor. These experiences dispose of one view of flutter and fibrillation which needs only passing mention with our present understanding of these processes, viz., the view that these conditions might be due to lost vagus control. We now know that the vagus is as active as normally. Robinson (1914) has even suggested that in fibrillation there is an increased susceptibility to vagus action (Weil).

Besides the well-known tendency of the vagus to cause slow conduction and to produce blocks, both of which effects are factors in the production of fibrillation by the mechanism of circus motions, there is still one other feature of its action which comes into play, namely, the distinct shortening of the refractory period. Observed by Walther (1899) after muscarin poisoning, the fact was used by him to explain the tetaniform contractions of the heart muscle obtained by him in this condition. Samojloff (1910) reported the shortening of the electrical disturbance after vagus stimulation, and Dale and Mines (1913) also showed that this electrical change corresponds to the change in the refractory period. Rothberger and Winterberg (1914) invoked shortening of the refractory phase as an essential part of their simple tachysystole theory of fibrillation. While a short refractory period is not essential to the theory of circus motions (Garrey), it has important bearings which have been emphasized by Mines, de Boer and Lewis. A short refractory period is one condition which keeps the ring-circuit open for reëntrant impulses and assists in maintaining a continuous circuit. Lewis, Drury and Bulger found that vagus stimulation shortened the refractory period to one-fifth or one-sixth the normal. This makes rapid "reëxcitation" possible with local faradization where localized vagus endings are stimulated with the muscle. It also makes it possible for circuits of small diameter to be established locally. Such vagus effects cannot be continually effective clinically. Finally, these authors make the further statement that because it shortens the refractory period, vagus stimulation may actually remove block developed in the course of vagus depression of conductivity. This is one of the unexplained paradoxes.

THE CIRCULATION IN AURICULAR FIBRILLATION. Gesell states that in auricular fibrillation, the ventricular volume output is increased above that maintained by venous pressure alone; he concludes that there are regular (650 per minute) coördinated systoles of small and variable amplitude which are really mechanically efficient and augment ventricular filling and output. Wiggers' (1915) accurate methods revealed no pressure changes attributable to coördinated systoles when the auricles were in true fibrillation. We may draw the inference that incoördination and ineffective auricular systoles such as unquestionably exist in auricular fibrillation interfere with ventriculr filling and output.

In a simple, uncomplicated case of auricular fibrillation, we have to deal primarily with two effects upon the ventricle which materially

jeopardize its mechanical efficiency, viz., the excessive rate and irregularity. Y. Henderson has shown that rates above a certain limit, even when regularity is maintained, will shorten the ventricular filling period and will decrease the volume output. While cases of auricular fibrillation show a great variation in ventricular rate, which may be as low as 30 in cases of bundle-block, the average lies between 110 to 140 per minute (Mackenzie, Lewis); it may reach 200 (Lewis). One type of so-called paroxysmal tachycardia probably is the result of regional fibrillation of the auricle with ventricular rates of 300. In these cases, it is well recognized clinically that there is a fall in arterial blood pressure and a rise in venous pressure (Hooker and Eyster, 1908), with cyanosis and edema. The heart, often pathological to begin with, suffers materially, not only as a result of the shortened rest period but because the general circulatory inefficiency spells inefficient coronary blood supply; there follows dilatation, relative valvular incompetency and the familiar picture of cardiac failure which, according to Lewis and Mackenzie, causes 70 per cent of all the cases of serious heart failure met with in practice.

The results of experiments confirm the observations of the clinic. From the very earliest experimental work, we learn that the auricle becomes unable to expel the blood entering it during the period of fibrillation; the auricles therefore dilate and present the appearance of diastolic rest, so much so that the condition was confused with and called "paralysis of the auricle" (Mackenzie, Hering). Lewis (1912) came to the conclusion that in auricular fibrillation, all circulatory disturbances observed by him were attributable to alterations in ventricular *rate*. The usual effects noted by him were decreased heart (ventricular) volume, decreased cardiac output, a rise in venous pressure, and a variable fall in arterial pressure which, however, may be compensated by other regulatory mechanisms. Wiggers (1915) found irregular intra-ventricular pressures depending chiefly upon the variable intervals between ventricular systoles, some of which, however, were so weak as to produce no heart sounds. Eyster and Swarthout (1920) recognize not only the result due to auriculo-ventricular interference and increase in rate, but those due to the factor of *irregularity* as well. In the fine type of fibrillation, they state that "Many of the ventricular beats are so premature, that the pressure does not rise sufficiently to open the semilunar valves." Eyster and Swarthout show that both flutter and fibrillation produce a variable reduction in the volume output of the ventricle, depending upon the development of the ab-

normal rhythm. The effect in fibrillation is greater than in flutter and is mainly due to the irregularity of the ventricle and to the high percentage of premature and abortive ventricular beats, rather than to the fast ventricular rate. Robinson (1914), studying a case of auricular fibrillation, concluded that exact estimations of blood pressure are clinically impossible due to the irregularity and variations in force and effectiveness of ventricular contractions. He gives approximate (high) figures for this particular case; the systolic pressures varied from 215 to 125 mm. Hg with the diastolic slightly more constant, between 120 and 85 mm. Hg. Cohn also maintains that blood pressures taken in the usual way are valueless, the range of both systolic and diastolic pressure is wide and fluctuating, so that average figures, such as those of James and Hart and of Kilgore, are of little significance. Cohn and Lunsgaard used Garten's method on a smaller vessel where the fluctuations are less. The readings are lower than for the brachial by 10 to 20 mm. Hg.

The tests of circulatory efficiences in auricular fibrillation show a decrease in vital capacity (Stewart, Harold and Carter, 1922) which Hewlett finds grades with the degree of decompensation. It increases with restoration of normal rhythm due to quinidine and in spontaneous normal intermissions. The former authors also show a marked improvement in blood gases both in line of oxygen saturation and coefficient of utilization.

Thus both experimental and clinical experience indicate the desirability of restoring cardiac efficiency by adopting measures to reduce the ventricular rate and to establish such a degree of regularity that the premature contractions super-induced by the fibrillating auricles shall be reduced to a minimum. Experimental procedures to accomplish this have been mentioned above. To discuss the therapeutic measures for its accomplishment, including the action of digitalis (Robinson, 1922) and quinidine would be beyond the scope of this review.

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THE FUNCTIONS OF THE GALL BLADDER

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The justification for a review of the work dealing with the functional significance of the gall bladder lies not so much in what is actually known concerning the functions of this exceptional organ, but in what has been imagined concerning its functions. In this attempt to separate fact from fancy, only experimental investigations and clinical observations, which have been made in a trustworthy manner, have been considered.

EMBRYOLOGY. It has been considered that the gall bladder arises from the same group of cells that gives origin to a portion of the liver. However, it probably has a more specific origin (83). The most important embryologic consideration, from a physiologic point of view, is that the gall bladder is formed from the group of primitive cells which also gives origin to the liver, pancreas, stomach and duodenum. From the embryologic standpoint, it would seem that the gall bladder is of some importance. Its phylogenesis also suggests that it is important because the autogeny of the gall bladder is established very early (16).

Comparative embryology of the gall bladder. While the embryology of the gall bladder has been worked out in relatively few species of animals, some of the species studied do not have a gall bladder in adult life: the Petromyzon, pigeon and rat (84). The history of the gall bladder varies markedly in each of these three forms. In Petromyzon there is a complete biliary apparatus which persists for a time and then totally degenerates. In the pigeon the gall bladder develops apparently in a normal manner, and then disappears. In the rat the gall bladder never develops. It is obvious that with the present data no conclusions can be drawn from the comparative embryology of the gall bladder with regard to its physiology.

ANATOMY. In order to appreciate the discussion of the possible functions of the gall bladder, a few phases of its anatomy should be considered. Although the size, position, relations and other charac-

teristics usually given in connection with the anatomy of an organ are of some importance, in reference to the physiology of the gall bladder, the histologic anatomy, the character of its blood, nerve and lymphatic supply, are of greater importance. It should be recognized at the outset that these structures vary greatly in the different species, and that the data of their comparative anatomy are at present far from being complete. An important anatomic fact with some physiologic significance is the relatively small capacity of the gall bladder as compared with the liver, and its volume of secretion. The position of the gall bladder varies greatly in different species, but it is usually in direct relation with the liver and often is partially or completely embedded in it. The ducts draining the gall bladder and liver also vary greatly, but they all have a variable relationship to the pylorus and pancreatic ducts.

The wall of the gall bladder is composed of four coats (87): *1*, mucous, *2*, fibromuscular, *3*, subserous, and *4*, serous, in the part covered by peritoneum. The mucous layer is composed of simple columnar epithelium which rests on an incomplete muscularis mucosa. The mucosa is thrown into a series of folds which form irregular pockets, the size of which decreases progressively from the fundus to the neck of the gall bladder. Mucous material is secreted by the cells of the mucosa, but goblet cells have not been reported. In the crypts formed by the folds of mucosa there are solitary lymph follicles. The fibromuscular coat is composed of smooth muscle fibers and interlacing bands of connective tissue. While certain observers have described a definite arrangement of the muscle fibers in layers, such an arrangement is difficult to find. The bundles of fibers of this coat interlace in all directions, the greatest number tending toward a transverse direction (39). The bundles of muscle and elastic connective tissue are very irregularly distributed. The subserous layer is composed of dense interwoven elastic tissue bands, and the serous layer of the typical peritoneal endothelial cells. In all the species in which the blood supply to the gall bladder has been determined, the blood comes from a definite main artery, and also from blood vessels from the liver. The course of the cystic artery is exceedingly variable in man (15), which is in accord with all the grosser anatomy of the gall bladder. The large blood vessels run in the subserous layer; the thickest plexus of capillaries is in the connective tissue layer of the fibro-muscular coat, just under the mucosa. The capillaries in this region are very numerous and especially thick in the folds of mucosa. The lymphatic

system draining the gall bladder is relatively large. The larger channels pass down the cystic duct, under the serous coat and drain into the adjacent lymph glands, communicating freely with the lymphatics of the liver. There are two sets of lymphatics in the gall bladder, a relatively poor one on the subserous layer, and a very rich one in the connective tissue just under the mucosa.

There are two types of nerves in the gall bladder, sympathetic ganglia with connective fibers, and medullated fibers accompanying the large arteries (20), (42).

Certain anatomic structures of the gall bladder and biliary ducts have been described, and while their functional significance has not been determined, they have been very intimately associated with the discussion of the function of the gall bladder.

The valves of Heister. Heister described certain folds in the cystic ducts of man which he believed functioned as valves. These have been studied by subsequent observers (Barker, Faure, Hartmann, Hendrickson, Terrier and Dally). Muscle fibers have been noted in these folds and they have been believed to be under nervous control. Their purpose has been assumed to be to prevent the entrance of bile into the gall bladder, or its exit from the gall bladder, depending on the viewpoint of the observer. They are not found in all species of animals. It would seem, regardless of their action and importance in some species of animals, that they cannot be considered of major importance with regard to the general physiology of the gall bladder.

The glands of Luschka. Luschka described certain structures in the mucosa of the gall bladder which have been designated as true glands, or simply hernias of the mucosa through defects of the fibro-muscular coat. It is questionable whether these are glands, although several theories have been promulgated with regard to their function (41), (24).

The cystic duct. Two points of possible physiologic significance must be considered in connection with the anatomy of the cystic duct which, in many species of animals, is very tortuous. Furthermore some of the older anatomists have described a sphincter, that is, an accumulation of bundles of more or less circular muscle fibers around the cystic duct (9), (39). These, together with the valves of Heister, have formed the anatomic basis for the explanation of the difficulty with which the gall bladder can be expressed by hand.

Parietal sacculi. Little saccular dilatations are found in the coats of the larger bile ducts, both intrahepatic and extrahepatic. They

have been termed glands of the ducts, and also little gall bladders appended to the ducts (8).

Comparative anatomy of the gall bladder. A method of obtaining data with regard to the possible function of an organ, which has been of value in some instances, has been used in relation to the function of the gall bladder, that is, a study of the comparative anatomy. In the study of comparative anatomists (75), (76) the biliary tract in the different species was observed primarily as a matter of routine in connection with the general field of comparative anatomy. The veterinarian anatomists (17), (85) have described the anatomy of the biliary tract in domestic animals only so far as it pertained to veterinary science. Certain observers (Dove, Hutchinson, MacAlister, Mann, Brimhall and Foster, and Rachford) made dissections and measurements of the biliary tract in various species of animals for the primary purpose of determining, from the comparative anatomy, whether or not conclusions could be drawn with regard to the function of the gall bladder. Data on the comparative anatomy of the biliary tract are not complete, and not only has the anatomy of the biliary tract in many species not been recorded, but there are many contradictory statements concerning some of the species which have been dissected and reported. One anatomist records the presence of the gall bladder in a certain species, while another asserts that the species is without a gall bladder. The whole field of the comparative anatomy of the biliary tract is being reviewed by Mentzer; his work, when finished, will be a valuable contribution to our knowledge of the anatomy of this important structure. A brief review illustrating the variability of the gall bladder in the different species of animals follows:

It has not been reported that the gall bladder exists in any lower form of animal life than fishes. Although a complete biliary tract develops in some of the cyclostome, it disappears before adult life is reached. In the Pisces the gall bladder is usually present, although there are well-established exceptions. Mentzer has collected from the literature eighteen species of fishes in which the gall bladder is absent. The gall bladder may be embedded in the substance of the liver or hang free from its surface, and in some species it is located quite a distance from it. The number and course of the bile ducts vary greatly. The bile may drain through hepatic ducts into the gall bladder and through the cystic duct into the duodenum, or various numbers of hepatic ducts may join the cystic duct to form the common bile duct. Hepatic ducts may enter the duodenum directly or enter the cystic duct. The cystic duct may drain into the stomach.

The biliary tract in the Batrachia has not been recorded in many species. The absence of the gall bladder has not been noted. Absence of the gall bladder in Reptilia has not been reported. The position of the gall bladder varies more in this species than in any other. It may be embedded in the liver or located at a relatively great distance from it. The hepatic ducts may drain into the gall bladder, cystic duct, or directly into the duodenum. In Aves the gall bladder is usually present, although there are notable exceptions. The hepatic ducts may unite with the cystic to form the common duct, drain into the gall bladder or enter the duodenum directly. In certain species the hepatic duct is dilated.

The greater variation with regard to the presence or absence of the gall bladder is found in the Mammalia. No Marsupialia have been reported in which it is absent. In the Rodentia the presence or absence of the gall bladder is rather variable; it is absent in many species. In both the Insectivora and Chiroptera the gall bladder seems to be invariably present. It seems to be absent in most of the Cetacea. In the Ungulata the presence or absence of the gall bladder is variable and in one family, the Perissodactyla, it seems to be uniformly absent. In the Carnivora and Primates the gall bladder seems to be present in all species. There is a wide variation in the relation of the various component parts of the biliary tract to each other, to the duodenum and to the pancreatic ducts in the mammals.

Several studies of the comparative anatomy of the biliary tract have been made primarily for the purpose of determining certain facts concerning the physiology of the gall bladder; the following are representative:

MacAlister (54) reviewed the work of the older anatomists with regard to the comparative anatomy of the biliary tract, and made many dissections of his own. He described the biliary tract in several representative species of each order of the higher animals. He considered the function of the gall bladder as being that of a temporary reservoir for bile. He tried to explain the presence of the gall bladder in some species and its absence in others as most probably related to the period of feeding. Animals that feed more or less constantly do not need a gall bladder, and consequently do not have one, while those that feed at intervals with more or less time between, need a gall bladder for storage. He points out that there are many exceptions to this, and it cannot be considered as the whole explanation.

Hutchinson (43), from studies at necropsy of the alimentary canal and its appendages in animals who died in the zoölogical gardens,

made several interesting deductions. He concludes that the gall bladder is almost functionless.

Rachford (78) made a rather extensive study of the comparative anatomy of the biliary tract, attempting to determine some of the reasons why the bil'ary tract, the pancreatic duct and the pylorus bore their respective relationships in different species of animals, as well as to account for the presence or absence of the gall bladder. He concluded that since bile is essential for the normal digestion of fat, the presence or absence of the gall bladder, the relation of the common bile duct to the pancreatic duct and to the pylorus, depends primarily on the relative amount of fat contained in the food.

Mann, Brimhall and Foster (63) dissected and measured the biliary tract in the common domestic and laboratory animals. Their data emphasized the fact that the biliary tract in the various species varied greatly, and that it seems impossible to explain the cause or purpose of these variations.

If the gall bladder is of any importance functionally, it is reasonable to assume that animals that do not possess the organ must have developed some means of compensating for the lack of it. The anatomic variation in the dimensions of the common bile duct has been considered a possible compensation. Mann, Brimhall and Foster collected data concerning the diameter and length of the common bile duct in animals with and without a gall bladder. The results obtained were variable, but did not seem to warrant the belief that there is any relation between the dimensions of the common bile or hepatic ducts and the presence or absence of the gall bladder. The comparison of a few species illustrates this point: The horse, which does not possess a gall bladder, has a relatively short duct with a large diameter; the ox, which possesses a gall bladder, has a duct which, although narrower, is much longer, and its relative capacity is almost as great as that of the horse. The same comparison can be made with regard to the deer and the sheep or the goat. The relative capacity of the extrahepatic biliary system of the deer, which has no gall bladder, certainly appears to be no greater than that of the sheep or goat, exclusive of the gall bladder capacity in the two latter species. In the horse and deer the hepatic duct is comparatively short, but its diameter is large as compared with closely related species having a gall bladder. The rat and pocket gopher, however, species without a gall bladder, have, as a rule, actually longer ducts with narrower lumina, as compared with such species as the guinea pig, rabbit and striped gopher, all of

which have gall bladders. In the latter species the comparable ducts are relatively no greater than in the species in which the gall bladder is absent. Thus it would seem that the relative capacity of the extrahepatic biliary tract is little, if any, greater in species of animals without a gall bladder than that of the same tract in comparable species which possess a gall bladder, and it does not, in any of the species, compensate for the lack of a gall bladder.

The relation between the pylorus, the entrance into the duodenum of the pancreatic ducts and bile duct, has been considered of importance as showing a relation between the function of the gall bladder and the digestion of certain foods, particularly fat (78). Mann, Brimhall and Foster (63) were not able to find a common factor in animals with a gall bladder and those without one, on the basis of a relationship of the pylorus to the point of discharge of bile. Examples may be cited as follows: The hepatic duct of the horse enters the duodenum between 10 and 20 cm. from the pylorus, while that of the ox enters between 50 and 70 cm. from the pylorus. On the other hand, in the rabbit the ducts enter the duodenum at about 0.5 to 1.5 cm. from the pylorus, in the guinea pig at 0.4 to 0.8 cm., in the rat at 1.5 to 2.5 cm., and in the pocket gopher at 4 to 5 cm.

Mann, Foster and Brimhall made a comparative study of the relationship of the common bile duct to the pancreatic duct in twelve species of animals possessing a gall bladder and four without a gall bladder. No definite connection between the presence or absence of the gall bladder and the relationship of the ducts was found.

A review of the data on the comparative anatomy of the biliary tract brings out many interesting facts, but the value of such data from the physiologic standpoint has not been of such positive value in indicating the function of the gall bladder, as of negative value in indicating that many of the theories concerning the general function of the gall bladder are untenable, because they are based on anatomic considerations only applicable to a small group of species. While the gall bladder may vary in function or functions, depending on the species of animal, its general function must be the same in all species. Therefore, any theory which will explain the general function of the gall bladder must be capable of being correlated with the variations of its anatomy. Thus far all attempts to correlate variations in the relation of the component parts of the biliary tract to each other, and the presence or absence of the gall bladder with a common factor have failed.

THE SPHINCTER OF THE COMMON BILE DUCT. It has been asserted that the sphincter of the common bile duct bears no relation to the function of the gall bladder (19); but it is certainly impossible to discuss the work on the physiology of the gall bladder without also considering the sphincter.

The anatomy of the sphincter. Gage (29) was the first to describe a sphincter at the duodenal end of the common bile duct. His description of such a structure in the cat is very complete. Oddi (71) made the first comparative study of the anatomy of the sphincter, employing several species of animals. He was able to demonstrate a definite sphincter in each species studied. Hendrickson (39) in his complete study of the anatomy of the biliary tract in the rabbit, dog and man, noted a definite sphincter of the common bile duct in each of these species. Helly (38) studied the end of the intramural portion of the duct in man, and concluded that a sphincter was present. Mann (60) studied the sphincter in ten species of animals possessing a gall bladder and four species without a gall bladder. Considerable difference was found with regard to the course of the ducts through the duodenal wall, and in the arrangement of the muscle fibers around the duct. A definite arrangement of muscle fibers which might function as a sphincter was found in each species studied, with no apparent difference in animals with and those without a gall bladder. Mann and Giordano (66) noted a group of muscle fibers at the end of the common bile duct in man which could act as a sphincter. Auster and Crohn (5) were not able to demonstrate a distinct anatomic sphincter, although they describe bands of smooth muscle passing around the intramural portion of the common bile duct in man and dog. The evidence is sufficient to permit the conclusion that there is a bundle of muscle surrounding the end of the common bile duct which could act as a sphincter. It is true that it is often difficult to find muscle fibers which encompass the duct exclusively, because they are so closely intermingled with the circular fibers of the intestine, and in many instances there are but few muscle fibers which might act exclusively as a sphincter. In certain instances what would appear to be the sphincter is only an accentuation of the muscle coat in the intramural portion of the duct over that of the extramural portion, by the addition of fibers from the circular coat of the duodenum. Often the interlacing of the muscle fibers passing around the duct, and the muscle fibers of the duodenum, is so intimate that it is hard to imagine a separate action of the two groups of fibers. Usually, however, there

are definite bundles of muscle which are distinct enough to be considered as a sphincter.

The tonicity of the sphincter in animals possessing a gall bladder. The anatomic demonstration of a bundle of small muscle around the termination of the common bile duct led to attempts to determine whether or not this muscle was sufficient to be of physiologic importance. Oddi (72) was the first to attempt to determine the pressure in the common bile duct which the sphincter would withstand. The method which he used on dogs, has been followed with slight variations by most of the subsequent investigators. It consists in section of the common bile duct, and inserting a cannula pointing toward the exit of the duct. A manometer is connected to the cannula. The duodenal wall is sectioned opposite the opening of the duct, exposing the papilla. Mercury is poured into the manometer until it appears in the aperture of the ampulla. The length of the column of mercury at this time is taken as a measure of the tone of the sphincter, and is found to be an equivalent of about 675 mm. water. Archibald (3) employed the same method except that he used water instead of mercury, and found that the sphincter in the dog withstood a pressure of 180 to 330 mm. water, and when acid (HCl) in concentration over 3 per cent was applied to the papilla, the maximal pressure withstood might be as high as 800 mm. water. Mann (59) determined the tone of the sphincter in six species of animals possessing a gall bladder: dog, cat, goat, rabbit, striped gopher (C. tridecemlineatus) and guinea pig. He found a marked variation in the pressure which seemed to measure the tone of the sphincter in the different species and in individual animals. However, the sphincter in each species of animal possessing a gall bladder withstood a minimal pressure of 75 to 100 mm. water. Reach obtained evidence of tone of the sphincter in guinea pigs, and noted the effect of drugs on it. McWhorter (56) noted marked variation in the pressure withstood by the sphincter in dogs; in some animals it was as high as 580 mm. water, and in others as low as 50 mm. water. Jacobson and Gydesen (44) concluded that 150 mm. of water was about the maximal normal pressure withstood by the sphincter.

It should be recognized that conclusions must be very cautiously drawn from the results of experiments of this nature, because of the many sources of error. All the experiments were necessarily complicated by an anesthetic and operative manipulations. The pressure taken as a measure of the tone of the sphincter might be due to many other factors, such as friction, peristalsis or contraction of the circular

muscle coat of the duodenum, and stretching and kinking of the duct. Undoubtedly in those experiments in which a strong irritant was employed, contraction of the wall of the intestine was a complicating factor. However, after all the possible sources of error have been considered, there is definite physiologic evidence of a sphincter at the end of the common bile duct which can withstand a small, but measurable pressure in the duct.

The tonicity of the sphincter of animals lacking a gall bladder. The tone of the sphincter in animals normally lacking gall bladders has only been tested in two species. Mann (59) tested it in the rat and pocket gopher (G. bursarius), neither of which has a gall bladder, and found that either no pressure was maintained by the sphincter, or at most only a very slight pressure, usually no more than 30 mm. water. Although definite conclusions cannot be drawn, owing to the small number of species employed, it would seem that animals without gall bladders do not have functioning sphincters.

The physiologic action of the sphincter. Doyon (22) noted relaxation of the sphincter at the time the gall bladder contracted. This would postulate a reciprocal action between the gall bladder and sphincter. Such a relationship has not been proved. The sphincter is also supposed to be affected by the gastric contents pouring into the duodenum. The physiologic mechanism, if there is such, regulating the action of the sphincter of the common bile duct has not been definitely determined. Many physiologic and clinical hypotheses concerning the gall bladder await the demonstration of such a mechanism.

ABSORPTION FROM THE GALL BLADDER. The difference in the bile in the gall bladder from that coming from the liver has long been recognized. Kemp noted the inspissated character of the contents of the gall bladder. Bidder and Schmidt (10) showed experimentally that much fluid is absorbed while the bile remains in the gall bladder. The results of all analyses demonstrate that bile of the gall bladder contains many times more solids than the bile coming directly from the liver, and that these solids are for the most part constituents of the liver bile. These data would definitely show that bile in the gall bladder has concentrated. On this basis Hohlweg (40) assumed that, by its absorption of water, the gall bladder could concentrate the bile secreted during the intervals between digestion; that is, the 40 cc. of bile found in the gall bladder represented from 240 to 400 cc. of bile from the liver.

The most conclusive evidence on this great absorptive power of the gall bladder, and the only quantitative study on this particular phase of its activity, has been reported by Rous and McMaster (82). In an ingenious series of experiments, they demonstrated that the gall bladder might concentrate the pigment reaching it to more than ten times its entering strength. They took advantage of the fact that in the dog the relation of the component parts of the biliary tract to each other is such that a ligature could be placed on the common bile duct so that the bile secreted by a portion of the liver would go into the ducts which were connected with the gall bladder, while the bile secreted from the rest of the liver passed into the bile duct to which a rubber balloon was attached. Thus the bile as secreted from the liver was separated into two divisions, one of which passed directly into a rubber bag, while the other was allowed to pass into the gall bladder. The pigment content of the bile was used as an index to determine the amount of absorption by the gall bladder. Control observations were made on the uniformity of pigment concentration of the bile secreted by the various portions of the liver, with regard to the possible influence of pressure in the gall bladder system of ducts, and other possible sources of error. It was found that the pigment content for each cubic centimeter of bile in the gall bladder might be ten times greater than the pigment content in the bile collected in the normal dog, showing that the bile in the gall bladder had been greatly concentrated. Other investigators (Harer, Hargis and Van Meter) demonstrated the absorptive action of the gall bladder by injecting potassium-sulpho-cyanide into the gall bladder, and testing the lymph returning with ferric chlorid. They obtained positive tests soon after filling the gall bladder with the solution. Boyd (14) studied absorption from the gall bladder by employing the prussian blue reaction. He noted absorption within half an hour. Virchow (90) described a definite accumulation of fat droplets in the cells of the mucosa of the gall bladder. Sudler (87) did not observe this. Aschoff (4) demonstrated that the epithelial cells of the gall bladder can take up neutral fat from the content of the gall bladder. Policard (77) concluded that fat absorption entirely comparable to the process in the intestine takes place in the gall bladder. Boyd described a lipoid in the mucosa of the dog and cat, but did not find any in the cow, rabbit or guinea pig. All data are in agreement that the gall bladder has great power of absorption. Most of this activity seems to be exerted in absorbing the water and thus concentrating the bile contained

within it. However, other substances are readily absorbed, and normally, possibly such substances as cholesterol are absorbed.

THE SECRETION OF THE GALL BLADDER. While the absorbing action of the gall bladder may account for most of the changes in its bile, undoubtedly the mucosa of the gall bladder also adds something to the contents, although it is probably only mucus. Birch and Spong (12) report interesting results of observations on a fluid obtained from a sinus draining the gall bladder in two cases in which it was believed the cystic duct was completely closed, and that the secretion of the gall bladder was draining to the surface. The amount of secretion was more than 20 cc. in twenty-four hours. It was viscid, almost colorless, alkalin, contained mucin, albumin and some inorganic constituents. It must be granted that the gall bladder may elaborate a specific secretion, but at present there is no evidence of such a function.

THE MOTOR MECHANISM OF THE GALL BLADDER. One of the earliest functions ascribed to the gall bladder was that it served as a reservoir for bile during the periods between feedings. In connection with such a function a motor mechanism whereby the gall bladder could empty itself would seem to be essential. The smooth muscle fibers in the wall of the gall bladder form an anatomic basis for this action. These fibers seem to be present in all species examined, although data concerning this are not complete. In any event the muscle coat must be extraordinarily thin in some species. The action of this muscle is a crucial consideration with regard to the function of the gall bladder: does the muscle empty the gall bladder or simply aid in preventing distention?

Physiologic consideration of the nerve supply to the gall bladder. The nerve supply to the gall bladder has been subjected to considerable investigation. Such studies are important, not so much from the anatomic standpoint, as from the bearing they have on the action of the gall bladder, and on the methods of studying the character and force of its contraction. The results of these investigations are not wholly in accord (36), (22), (51), (18), (27), (6), (52). Heidenhain and Doyon (36) believed that the splanchnic nerves were motor to the gall bladder. Freese (27) concluded that the splanchnics contained both motor and inhibitory fibers. Langley (51), Bainbridge and Dale (6), Lieb and McWhorter (52) found the splanchnics to contain inhibitory fibers. Doyon (22) was unable to obtain any effect on the gall bladder by stimulation of the vagi. Courtade and Guyon (18), Bainbridge and Dale (6), and Lieb and McWhorter (52) concluded that

motor fibers passed through the vagi to the gall bladder. The discrepancy in the results are probably due, as Bainbridge and Dale point out, to the fact that as the earlier investigators left the gall bladder in apposition to the liver, the results they obtained were owing to changes in liver volume, and not to contraction of the gall bladder. These investigators separated the gall bladder from the liver, while Lieb and McWhorter used the isolated gall bladder and drew their conclusions from the action of drugs. In all probability, as Meltzer (68) suggests, there is a contrary innervation of the gall bladder. As is seemingly true with the other viscera, all the motor and inhibitory fibers do not run in the same nerves, but some of each are found in the vagus and splanchnic; nevertheless the vagus is mainly motor, and the splanchnic mainly inhibitory to the gall bladder.

Movements of the gall bladder. While many previous observations have been made on the contractility of the biliary passages, Doyon was the first to record graphically the rhythmic contractions of the gall bladder. He introduced a balloon filled with water into the fundus of the gall bladder and connected it with a piston recorder. He was able to show that the gall bladder underwent spontaneous, slow, and rather irregular contractions. Bainbridge and Dale found that the gall bladder of the dog underwent spontaneous contractions, varying in rate from one to three each minute. Okada (74) passed a rubber balloon fastened to a glass tube into the gall bladder through a previously made fistula, and attached a water manometer and piston recorder to the bottom. By this means the movements of the gall bladder could be recorded, and observations made without any anesthesia or narcotic. Okada noted spontaneous contractions occurring two to five times each minute. These contractions might start or stop without apparent cause. He noted a great increase in the rhythmic contractions after a meal of meat. Mann and Giordano (66) noted rhythmic variations in pressure in dogs with a permanent gall bladder fistula, which could be attributed to movements of the gall bladder. Winkelstein (94) sewed four very small, flat disks of silver in a quadrilateral arrangement on the serosa of the ventral wall of the gall bladder, and then studied the relative position of these metal disks by the x-ray. He was not able to determine any movement of the gall bladder other than indirect ones due to respiration. Friedenwald, Martindale and Kearney (28) did not observe a contraction of the gall bladder in anesthetized animals. However, the general evidence indicates that the gall bladder undergoes spontaneous rhythmic contractions, although

they are probably not marked and may quite easily be masked by other concomitant changes. The functional significance of such movements are not known.

The force of contraction of the gall bladder. Since the gall bladder has muscle fibers in its walls and can thus be considered as a muscular viscus, it became of importance in connection with its possible function to determine the maximal pressure the muscle could exert on the contained fluid. Although Doyon first graphically recorded the movements of the gall bladder, it is not possible from his results to determine the maximal force of its contraction. This is also true of the reports of most investigators who studied the movements of the gall bladder, its nerve supply or the action of drugs on it (6), (18), (36), (51), (52). Freese (27) made a special study of the force of contraction of the gall bladder and found that after stimulation of the nerve leading to it in the anesthetized dog, a maximal of from 216 to 313 mm. water pressure was produced. Okada (74) noted that in his dogs with a permanent biliary fistula the gall bladder contracted against pressure greater than 300 cm. water. Mann and Giordano did not note rhythmic movements of pressure within the gall bladder when such pressure was greater than 340 mm. water.

Measurements have never been made of the force of contraction of the gall bladder that are totally free from objections. There seems to be no doubt that the gall bladder has a contractile mechanism which exerts pressure on its contents, but the contractile power of its own intrinsic muscles does not seem to be great, and in all probability it cannot exert a pressure greater than the secretory pressure of the liver.

The filling of the gall bladder. The manner in which the gall bladder is filled, the time during which bile enters it, and other factors with regard to the passage of bile into it are not known, and very little work has been done on the subject. Auster and Crohn (5) studied the appearance of phenol-tetrachlorphthalein in liver bile and in gall bladder bile. They observed the dye in the bile discharged into the duodenum, and in the gall bladder bile at about the same time, ten to fifteen minutes after administration.

The emptying of the gall bladder. One of the most debated problems in connection with the action of the gall bladder is whether or not it actually empties, and if so how, and in what time. Opinions vary: it is believed by some that the gall bladder is considered capable of emptying in the same manner as the urinary bladder, and by others

that anything which enters the gall bladder does not pass out of the cystic duct. Auster and Crohn, in acute experiments, injected methylene blue into the gall bladder of dogs, and were unable to produce an expulsion of the stained contents. They also placed suspensions of carmine powder or charcoal in the gall bladder aseptically, and after varying periods the animals were killed. Stasis of the injected substance up to three days was observed, but the gall bladder emptied within one week. Winkelstein (93) found that indigocarmin remained in the gall bladder of a dog from four to five days, but was discharged at the end of two weeks. Diamond (19) injected a carmine suspension into the gall bladder. A closed duodenal fistula was then made and the discharged bile examined at various periods for the carmine. Carmine granules in the gall bladder were found six weeks after injection. Johnson (45) was not able to show that the gall bladder emptied itself. Many surgeons have remarked that the gall bladder is never found empty at the time when the abdomen is opened. At necropsy of both man and animals the gall bladder is never empty. The evidence is quite conclusive that the gall bladder never completely empties at one time. It appears to empty slowly, and not in a manner similar to the urinary bladder. It should be noted that the experimental work on this problem has two sources of error; some of the experiments were necessarily complicated with an anesthesia, and all were complicated with operative procedures.

DISCHARGE OF BILE INTO THE DUODENUM. Numerous investigations have been made with regard to the rate of bile secretion and the various factors, such as food and drugs, which might change it. The point of interest in this relation to the possible function of the gall bladder is the rate of bile discharge into the intestine. This rate of discharge would depend on the rate of production by the liver, influenced by whatever effect, if any, the gall bladder and the sphincter of the common bile duct might have upon it. An analysis of the rate of discharge of bile might give some indication of the function of the gall bladder.

Discharge of bile into the duodenum in animals with a gall bladder. It is a matter of common observation that the bile of laboratory animals is discharged into the intestine intermittently. Such observations have usually been made in the course of other experiments, always complicated with an anesthetic. Rost (81) made extensive investigations on dogs with permanent duodenal fistula. He placed a specially constructed cannula in the duodenum which made it possible to observe the discharge of bile from the papillary orifice. He noted that in

the normal fasting dog, bile was seldom discharged into the duodenum oftener than once an hour. A strong irritation was necessary in the normal dog to cause a flow of bile. Among several substances used to increase the rate of discharge of bile, peptone was the most active. The injection of peptone solution into the duodenum was followed in less than a minute by a gush of dark brown bile. Such evacuation was repeated two or three times during the next few minutes and was arrested after fifteen minutes. If the peptone solution was again administered, the discharge of bile was considerably less than after the first administration. Rost attributed this intermittent discharge to the opening of the sphincter of the common bile duct, and the expulsion of bile from the gall bladder. Klee and Klüpfel (50) also investigated the discharge of bile into the duodenum in normal dogs, using the Rost method, and their results are almost identical. They observed almost no discharge of bile into the duodenum of a normal fasting dog during a period of from one to two hours, and a flow of bile beginning one to three minutes after taking food, a psychic secretion. When the gastric contents were expelled into the duodenum, as every peristaltic wave from the stomach passed the pylorus, a flow of bile occurred. The injection of peptone solution into the duodenum invariably produced a discharge of bile.

The evidence indicates that in animals normally possessing gall bladders, the liver secretes more or less continuously, although the rate varies enormously. The rate of discharge of bile into the duodenum, however, is intermittent.

Discharge of bile into the duodenum in animals normally lacking a gall bladder. Very few observations have been made on the rate of discharge of bile into the duodenum in species of animals normally lacking a gall bladder. Mann (59) did not observe an intermittent rate of discharge in two such species, the rat and pocket gopher. These observations were necessarily made while the animal was under an anesthetic. The data are too few, but in all probability animals normally lacking a gall bladder discharge bile continuously into the duodenum.

THE COMPARATIVE PHYSIOLOGY OF THE EXCRETORY FUNCTION OF THE LIVER IN RELATION TO THE GALL BLADDER. Very little work has been done to demonstrate a possible difference in the excretory function of the liver of animals possessing a gall bladder as compared with animals in which it is absent. Mann and Foster (65) did not find any difference in the secretory pressure of the liver in animals with

a gall bladder and those without. McMaster (55) noted that the liver of the rat (gall bladder absent) secreted bile about eight times higher in pigment than the mouse (gall bladder present).

EFFECT OF REMOVAL OF THE GALL BLADDER. One of the valuable means for attempting to determine the function of an organ is to study the effects of its removal. This has been done many times with regard to the gall bladder, and the results form probably the most important data concerning its functional significance. The gall bladder has been removed experimentally from several species of animals, and it has been removed from man a multitude of times for pathologic conditions.

Effect of cholecystectomy upon the biliary ducts. Zambeccari (95) is credited with performing the first cholecystectomy, although there are vague references in the literature of previous extirpations of the gall bladder, or ligation of the cystic duct. There are many incomplete reports of subsequent experiments dealing with cholecystectomy, but Oddi (73) was the first to remove the gall bladder for the purpose of studying the effect of such a procedure on the possible function of the organ. He noted a dilatation of the cystic, hepatic and common bile ducts to twice or three times their normal caliber following cholecystectomy. These experiments have been repeated by many investigators (De Voogt, Eisendrath and Dunlavy, Haberer and Clairmont, Hartman, Smyth and Wood, Hautefort, Hohlweg, Judd and Mann, Nasse, Rosenberg, and Rost), and with the exception of Hohlweg, Nasse, and Rosenberg obtained results identical with those of Oddi (73). Many species of animals have been employed, including the dog, cat, sheep, goat, rabbit and guinea pig. Many surgeons (2), (23), (26), (33), (46), (47), (81), (86) have also noted a dilatation of some portion of the biliary tract in man after cholecystectomy. Sweet observed marked enlargement of the parietal sacculi following cholecystectomy. The evidence, both from experimental investigation and clinical observation, definitely proves that removal of the gall bladder is usually followed by a dilation of the remaining extrahepatic biliary tract. If the stump of the cystic duct has been left long, this structure may dilate in a pear-shaped manner until its capacity may equal one-tenth the capacity of the original gall bladder. If the cystic duct is completely removed, only the remaining extrahepatic biliary tract dilates. The intramural portion of the common bile duct does not dilate to any extent, nor does dilatation occur in the ducts within the liver itself. The dilatation is most pronounced where the tissue surrounding the

ducts is least. Rarely removal of the gall bladder is without effect on the remaining biliary tract.

Effect of cholecystectomy on the tonicity of the sphincter of the common bile duct. Judd and Mann (48) studied the tone of the sphincter of the common bile duct at various periods after cholecystectomy. They found that the tonicity of the sphincter was abolished or greatly reduced after removal of the gall bladder. Conversely they destroyed the muscle fibers around the intramural portion of the duct and found that the ducts then were not affected by the cholecystectomy. It thus would seem that the dilatation of the ducts following removal of the gall bladder depends on the activity of the sphincter of the common bile duct.

The discharge of bile into the duodenum after cholecystectomy. Rost (81) studied the discharge of bile in cholecystectomized dogs with permanent biliary fistula. He found that the discharge after removal of the gall bladder was not the same in the different animals, and varied with the length of time after operation. There was more or less constant dribbling of bile into the duodenum immediately after the gall bladder was removed. A very slight irritation caused a small gush of bile to be discharged. In certain dogs this constant flow of bile following cholecystectomy did not cease even after many months. In others the intervals between the discharge gradually lengthened until they reacted like normal dogs. The injection of peptone into the duodenum did not cause a discharge of bile. Klee and Klüpfel (50), employing the same method as Rost, obtained similar results with regard to the flow of bile following cholecystectomy. In one dog bile dribbled for six weeks, and then ceased and was discharged as in a normal animal. In another dog, an intermittent flow did not occur during the period of observation after operation.

The evidence presented indicates that after removal of the gall bladder the discharge of bile into the duodenum is more or less continuous in all animals. In certain cases in man the rate, after a variable period of time, may become intermittent, while in others it always remains continuous.

Effect of cholecystectomy on other functions. That removal of the gall bladder may be without any demonstrable effect on health and general well-being is attested to by numerous operations on human beings and some experimental data. Mann (58) observed a dog for three and one-half years after cholecystectomy and did not note any change in nutrition and general condition. Hohlweg (40) obtained a

distinct decrease in the acid content of the gastric juice in cholecystectomized patients and dogs. Others (13), (57) found normal acidity in the gastric juice after removal of the gall bladder in man. Gatewood (30) found normal acidity in dogs after cholecystectomy. Rost noted a decrease in pancreatic juice in his cholecystectomized dogs. Rosenberg (80) and Rost (81) did not find any changes in metabolism following removal of the gall bladder. Judd (46), (47) was not able to discover any effect of cholecystectomy on the health of patients. Afanassiew (1) injected wax into the biliary tract and noted that jaundice developed more quickly than when only the common bile duct was occluded. Mann, Bollman and Magath (62) found that bilirubin appeared in a detectable amount in the blood stream after obstruction of the common bile duct many hours sooner if the gall bladder was also removed. Sweet (88) found that the cholesterol content of the blood was increased after removal of the gall bladder and did not reach normal for a period of forty days.

Without doubt loss of the gall bladder does not affect the general nutrition or metabolism to any noticeable extent. Whether or not it may influence the action of any other organ has not been determined. It does seem to affect the cholesterol and the rate of development of jaundice.

LESIONS OF THE GALL BLADDER IN RELATION TO FUNCTION. Very little of physiologic significance has been ascertained from a review of lesions of the gall bladder. While a true cholecystitis can be found in various species of animals, it seems to be much more common in man. In this connection the possibility that, when man assumed the erect posture, the position of the gall bladder in relation to the remaining part of the biliary tract was changed, must be considered. Mann (61) has demonstrated that a true chemical cholecystitis can be produced, showing that the gall bladder can be made to produce a specific reaction.

THE THEORIES OF THE FUNCTIONS OF THE GALL BLADDER. Several definite functions have been ascribed to the gall bladder. Usually such theories have been based on only one or two considerations, and possibly none of them expresses fully the physiologic significance of the organ.

The reservoir theory. Probably the first and most popular theory of the function of the gall bladder is that it serves as a reservoir for the bile secreted during the intervals between feedings. During the active digestive processes, bile is poured into the intestine almost as fast as it

is secreted; in the interval between digestive periods the bile secreted is stored in the gall bladder. It was soon appreciated that the gall bladder could not function as a reservoir in the same sense as the urinary bladder. W. J. Mayo (67) gives two reasons for this: first, the capacity of the gall bladder in relation to the amount of bile secreted is too small, being about one to forty in man; and second, the propulsive power of the gall bladder is not sufficient to empty it quickly. Mann (58), from the results of experiments on various species of animals, estimated that the capacity of the gall bladder was usually less than the amount of bile secreted in one-half hour. However, as Hohlweg pointed out, and the work of Rous and McMaster demonstrated quantitatively, the absorptive power of the gall bladder greatly increases its storage capacity, so that as regards capacity, it is possible to consider the gall bladder, to a certain extent, a reservoir. When the position of the gall bladder is considered in the relation to changes in pressure produced in the abdominal cavity by respiration, as occurs in many species of animals, it would seem that a small reservoir with large capacity due to concentration, would be highly desirable, if a reservoir was the thing desired.

The absorption theory. The changes that the bile undergoes in the gall bladder have led to the view that its main function is that of absorption. Sweet believes that the absorption of cholesterol is an important part of this function. There are many factors that would indicate that the gall bladder has an important absorptive function, the many lymphatics, the character of the mucosa and the movements could be taken as permitting closer relation of the absorbing surface of the contents. Whether this absorbing ability is only for concentrating the contents of the gall bladder, or is a specific, is unsettled.

The secretory theory. Although the mucosa of the gall bladder is histologically not of the type which is usually found on a secreting surface, it must be granted that it is possible the gall bladder adds something to its contents besides mucus. In this connection the movements of the gall bladder could be considered as mixing the product elaborated with the bile.

The bile flow regulator theory. There are two aspects to the theory that the gall bladder regulates the flow of bile. One conception is that the gall bladder makes the bile more viscid and thus regulates its passage through the bile duct (11). Neither the physical possibility nor the rationale for such a theory seems apparent. The other conception is that the liver secretes bile more or less continuously, but that

owing to sphincteric activity, the discharge of bile into the intestine is intermittent. This change from a continuous flow of bile into an intermittent one is made possible by the gall bladder. That some such process takes place seems plausible, but its functional significance is not evident.

The pressure regulation theory. The theory that the gall bladder acts as an expansible chamber regulating the pressure in the biliary tract has various phases. The simplest explanation of such a function is that it acts as a safety mechanism in relation to pressure changes incident to the respiratory movements (92). The respiratory movements may produce changes in intraduct pressure and the gall bladder may be concerned in these pressure changes; that this cannot be an important general function of the gall bladder is readily apparent when it is recalled that a well-developed gall bladder is present in most species of fishes. Other aspects of this theory are similar to the bile flow regulator theory.

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CHEMICAL CHANGES IN THE BLOOD AND THEIR CLINICAL SIGNIFICANCE

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The clinical use of chemical blood analyses as a measure of various metabolic and excretory processes has developed with startling rapidity during the past ten years, due largely to the introduction of numerous new analytical methods. Although the methods are new and the remarkable progress which has been made in this field is a consequence, the conception of employing chemical blood analyses in the study of disease is by no means new. In 1790 Lavoisier concluded his last scientific communication to the Académie with these words:

> Up to the present time we have learned only to conjecture as to the cause of a great number of diseases and as to the means of their cure. Before hazarding a theory we propose to multiply our observations, to investigate the phenomena of digestion and *to analyze the blood both in health and disease.* We will draw upon medical records and the light and experience of learned physicians who are our contemporaries, and it will be only when we are thus completely armed that we will dare to attack a revered and antique colossus of prejudice and error.

A hundred years ago (November 15, 1821) Prevost and Dumas read a now classic paper before the Society of Physics and Natural History of Geneva (published 1823) in which they demonstrated that when the kidneys were removed in cats and rabbits, urea rose to a high concentration in the blood. From this they concluded that urea was not formed in the kidneys and that waste products must accumulate in the blood when the kidneys fail to perform their excretory function. The analytical data which led to this deduction consisted in the gasometric analysis of urea nitrate which had been separated from the blood serum of animals whose kidneys had been removed. Excellent agreement was shown with analyses of urea nitrate prepared from urine. Dumas' method for the estimation of nitrogen is a sufficient guarantee of his analytical skill. This work of Prevost and Dumas was for many years the starting point of all studies on the so-called uremia encountered in the terminal stages of Bright's disease.

In 1848 Sir A. B. Garrod put the subject of the uric acid content of the blood on a definite basis when he identified this substance in the blood of patients suffering from gout, and showed that whereas uric acid is normally present in human blood only in traces, it is definitely increased not only in gout, but in certain cases of nephritis. He further noted that there is no increase in the blood uric acid in rheumatism, such as is found in gout, and used this as a point of differential diagnosis. It has been suggested that Garrod drew upon his imagination for some of his results, but the correctness of his deductions and the quantitative data which he gives do not support this. This criticism probably originated from the fact that in his later clinical work he endeavored to gauge the amount of uric acid present in the blood by his famous thread test ("uric acid thread experiment"). This test was checked against tests where known amounts of uric acid had been employed. In his earlier work, however, the uric acid (or urates) obtained from a given amount of blood, 65 cc. (1000 grains) was weighed. Such figures as the following recalculated as milligrams per 100 cc. were obtained: in gout—5, 5, 2.5, 4.5, 3.0 and 17.5; in rheumatism—trace in four and negative in fifth case; in albuminuria—0.5, 1.2 and 2.7 mgm. Sheep and pigeon blood yielded negative results.

That blood may contain a sugar-like substance was first recognized in 1775 by Dobson, who detected its presence in diabetic blood serum by its sweet taste. In the same year with Pool he had been able to separate sugar from diabetic urine. It remained, however, for Tiedemann and Gmelin in 1831 to definitely prove the presence of sugar in normal blood by applying the fermentation test to alcoholic extracts of blood. Their work marks a new epoch for they showed that the carbohydrate foods are broken down to sugar in digestion and absorbed into the blood, thus definitely proving that the blood sugar is a product of normal metabolism and not a pathological product as had formerly been supposed. During this period the subject of blood sugar occupied the attention of many investigators and was the topic of frequent controversies. The brilliant researches of Claude Bernard, chiefly between 1850 and 1860, did much to elucidate the mechanism of carbohydrate metabolism. He discovered the glycogenic function of the liver and by means of his sugar *piqûre* first noted the connection between hyperglycemia and glycosuria. Prior to the introduction of our present micro methods of blood sugar estimation we possessed relatively accurate data on the sugar content of the blood both in normal and pathological

conditions, as is evident from Bang's excellent monograph, *Der Blutzucker*, written in 1913.

No review of the chemical composition of the blood would be complete without reference to the classic analyses published by Carl Schmidt in 1850. In an effort to throw some light on cholera, Schmidt made very complete determinations of the inorganic constituents and the proteins of the plasma and corpuscles. His data also included analyses of the blood of several diabetics and nephritics, as well as figures for the blood of a normal man and woman. Regarding the last named, Bunge stated fifty years later (1902); "In order to give an idea of the human blood, I subjoin the analysis of my revered teacher, Carl Schmidt, one that has not been surpassed." Bunge (1876) devoted considerable attention to the composition of the blood of several species of animals in Schmidt's laboratory at Dorpat, but his data scarcely bear comparison with the monumental work of Schmidt. However, we find that in 1898 Bunge had transmitted the inspiration he received from Schmidt to his pupil, Abderhalden, who carried out the most complete analyses which we possess on the blood of the various domestic animals, especially as regards the mineral constituents.

Walter in his investigations on the action of acids in the animal organism, carried out in Schmiedeberg's laboratory in 1877, clearly recognized the relation which exists between the CO_2 content of the blood and the blood alkali. He showed that the venous CO_2 of rabbits could be reduced to approximately one-tenth its normal value by the injection of acids such as hydrochloric and phosphoric. Bunsen's method of gas analysis was employed. The clinical applications of this principle were clear, but owing apparently to analytical difficulties the method did not come into practical use until forty years later, when Van Slyke (1917) described his simple method of estimating the CO_2 of the blood.

It is apparent from the foregoing that many of the older workers recognized various pathological changes in the blood in disease, and correctly interpreted the conditions underlying them, but as Folin (1922) has remarked in this connection in an earlier review: "Their reasoning power was necessarily much superior to their analytical skill or their meager laboratory facilities, and their analytical data could have been little more than mere ornaments attached to good, logical reasoning." Nevertheless, the correctness in deductions of many of these workers is quite remarkable, and would indicate that the information obtained, even with their unsatisfactory analytical methods, was not entirely misleading. The fifty-year period, 1860–1910, which

covers many of the most fundamental and important studies in nutrition, witnessed few advances in the field of blood analysis. Rather there appears to have been a retrogression. Although Hoppe-Seyler in 1879 presented a quite comprehensive discussion of this subject in his text on physiological chemistry, very little is to be found in texts written as late as 1910. It would appear that, in view of the fact that many of the older statements had not received confirmation, discussion of the subject was gradually omitted.

During the past decade, 1910–1920, the chemical composition of the blood has been a topic of increasing interest and importance, quite eclipsing in significance the studies carried out on the urine during the preceding decade. In the case of the urine the advances were primarily the result of the impetus furnished by the new methods of Folin, supplemented later by those of S. R. Benedict, and these same workers, together with Van Slyke are responsible for many of our methods of blood analysis. To this list should also be added the name of the late Ivar Bang, who developed many very ingenious but less practical micro methods of blood analysis. The importance now attached to the chemical examination of the blood would appear to be rapidly overshadowing the importance formerly attached to the examination of the urine.

It is the object of the present review to consider various changes in the chemical composition of the blood, more particularly those which occur in disease and the interpretations which can be placed upon them. Within a remarkably short time the chemical analysis of blood has come to be used in the diagnosis, prognosis and treatment of disease. The subject holds much of interest alike to the physiologist and clinician. So many workers have been attracted to this field of study that references to the literature alone would fill the space allotted to this review. The reviewer is therefore confronted with the task of selecting and discussing those chemical studies on the blood which appear to him to have contributed most in the recognition and interpretation of pathological conditions.

Blood volume. Owing principally to the recent work of Keith, Rowntree and Geraghty (1915), the subject of blood volume has received considerable attention. These investigators have introduced a new method of determining blood volume and have obtained somewhat higher figures than those formerly given for man. The principle underlying their method is the introduction directly into the circulation of a non-toxic, slowly absorbable dye (vital red) which remains in the plasma long enough for thorough mixing, and the determination of its concen-

tration in the plasma colorimetrically by comparison with a suitable standard mixture of dye and serum. According to this method the plasma normally constitutes approximately 5 per cent, or one-twentieth of the body weight. The volume occupied by the corpuscles was calculated with the aid of the hematocrit and found to average 43 per cent for the cells and 57 per cent for the plasma. On this basis Keith, Rowntree and Geraghty have calculated that blood normally constitutes 8.8 per cent or 1/11.4 of the body weight. With this method they were able to demonstrate the amount of decrease in blood volume as the result of hemorrhage and of the increase following infusion of saline.

TABLE 1

Observations on blood volume (Bock)

CONDITIONS	NUMBER OF CASES	TOTAL PLASMA	TOTAL BLOOD	HEMOGLOBIN CALCULATED FOR O. CAPACITY	RED BLOOD CELLS
		per cent of body weight	*per cent of body weight*	*per cent*	*millions*
Normal	5	5.1	8.2	16.4	4.8
Polycythemia	3	5.1	13.7	22.5	9.1
Pernicious anemia	7	4.9	5.7	5.8	1.6
Miscellaneous	7	4.9	7.1	10.9	3.9
Diabetes	8	4.8	7.3	16.3	4.6

Significant observations were made in a few pathological conditions. Both the blood and plasma volume are increased in pregnancy, before term, but return to normal within a week or two after delivery. In obesity the plasma and blood volumes are relatively small. This has recently been confirmed by Brown and Keith (1924). Many cases of anemia exhibit a relatively high blood volume, while in some cases polycythemia in the sense of a high blood count may be dependent on a low plasma volume. In anasarca accompanying myocardial insufficiency the blood volume may be absolutely increased. In many cases of marked hypertension the volume is small, indicating that hypertension is not necessarily dependent upon a large blood volume.

The recent publication of Bock (1921) on blood volume presents some very interesting data obtained with the vital red method on five normal and twenty pathological cases. Bock's figures are given in table 1, the hemoglobin being recalculated to grams per 100 cc. The constancy of the plasma volume under widely varying conditions is pointed to as

a striking fact. Although the plasma volume remains practically normal in polycythemia and anemia, as shown by Bock's data, the total blood volume is increased in the former and decreased in the latter owing to variations in the cell content. Blood volume was the topic of a review by Erlanger in 1921.

Blood proteins. Normally the total solids of the blood amount to 19 to 23 per cent, of which all but about 1.5 per cent is protein. Of the whole blood proteins hemoglobin comprises nearly three-quarters, the plasma proteins one-quarter. From this it is evident that hemoglobin constitutes the most important variant in the solid content of the blood. In cases of severe primary and secondary anemia the total solids may be reduced to less than half their normal value as a result of reduction in the hemoglobin content.

The normal *hemoglobin* content of the blood of the human adult, males and females taken together, amounts to about 16 per cent. It seems unfortunate that most of the estimations recorded should have been made employing an empirical scale with 100 as the normal, especially since the 100 is somewhat of a variable factor with different methods owing to different standardizations. The hemoglobin content of the blood varies widely not only in disease, but also in different age periods, as pointed out by Williamson (1916). In the past one of the greatest difficulties in the way of making an accurate colorimetric hemoglobin estimation has been the securing of a correct standard. Van Slyke's (1921) method of determining the oxygen capacity of blood has solved this difficulty, while Newcomer (1919) has produced a permanent color standard for the acid hematin method in the form of a glass plate, which may be used with any colorimeter made on the Duboscq pattern.

The plasma proteins (*albumin, globulin and fibrinogen*) have recently received considerable attention at the hands of Epstein (1912), Robertson (1915), Rowe (1916–17), Cullen and Van Slyke (1920), Howe (1921), Wu (1922), Linder, Lundsgaard and Van Slyke (1922–23), Salvesen and Linder (1923) and others. Rowe has studied the serum proteins in normal and a number of different pathological conditions, employing Robertson's micro-refractometric method. In a series of twenty-two normal cases the serum albumin was found to vary between 4.6 and 6.7 per cent, the serum globulin between 1.2 and 2.3 per cent and the total serum proteins between 6.5 and 8.2 per cent, while the percentage of globulin in the total protein varied from 16 to 32 per cent. Muscular activity, even of the simplest sort, increased total serum proteins,

this increase occurring more in the albumin than in the globulin fraction. In three cases with severe muscular work Rowe found the total protein increased from 1.1 to 1.9 per cent and the albumin from 0.8 to 1.5 per cent, while in one case with light exercise the total protein was increased 0.5 per cent and the albumin 0.3 per cent. Linder, Lundsgaard and Van Slyke found the average normal values to be: total protein 6.73 per cent, albumin 4.12 per cent and globulin 2.61 per cent, the globulin constituting 39 per cent of the total protein. The highest normal value for total protein was 7.45 per cent and the lowest accepted 6.22 per cent. With his new colorimetric method Wu obtained the follow-

TABLE 2

Blood serum proteins in health and disease

Averages compiled from data of Rowe

CONDITION	NUMBER OF CASES	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN TO TOTAL FROTEIN
		per cent	*per cent*	*per cent*	*per cent*
Normal subjects	22	5.6	1.9	7.5	22.5
Syphilis	19	5.0	2.5	7.5	34
Pneumonia	8	3.7	2.5	6.2	40
Chronic nephritis with edema	3	2.5	1.7	4.2	40
Chronic nephritis with uremia	5	4.2	2.3	6.5	35
Chronic nephritis without uremia or edema	7	4.5	2.2	6.7	33
Cardiac decompensation	9	4.7	2.6	7.3	36
Arteriosclerosis	9	4.8	2.3	7.1	32
Diabetes	10	5.5	1.9	7.4	26
Anemia	9	3.9	1.7	5.6	30

ing averages for five normal individuals: total proteins 6.94 per cent, albumin 4.85 per cent and globulin 2.09 per cent, with a globulin percentage in terms of total serum protein of 33 per cent.

Table 2 compiled from data given by Rowe gives a comparative idea of the blood serum proteins in the normal human subject and a variety of pathological conditions.

In syphilis, according to Rowe, the globulin is definitely increased, while the total protein remains about normal. In pneumonia the globulin is increased more in relation to the total protein than in syphilis, while the total protein is reduced, due probably in large measure to a dilution of the blood serum by water retention, which occurs in fever. Rowe obtained the lowest values for total serum proteins in chronic

nephritis with edema and expressed the opinion that this is due to chronic intoxication as well as hydremia. In chronic nephritis with uremia the total proteins may be nearly normal but the globulin is usually increased. In pernicious anemia the total proteins are not as low as would be expected from examination of the whole blood, being higher than in nephritis with edema.

Pathologically the most interesting data in table 2 are found in nephritis with edema (nephrosis). Epstein called special attention to the changes in the blood serum proteins in nephrosis several years ago, pointing out that in this condition there is not only a reduction in the total protein, but also that the globulin is both absolutely and relatively increased, constituting as much as 90 per cent of the total protein in exceptional cases. Salvesen and Linder have recently reported observations on a case with a total protein content of 4.35 per cent, of which 77 per cent was globulin. These investigators have studied the relation between the serum calcium and the serum proteins in nephritis. They express the opinion that the moderate reduction in calcium found in non-uremic cases without phosphate retention is due to the reduction in the serum proteins to which the non-diffusible calcium is bound. In kala-azar Wu has found a condition in which the total serum proteins may be increased to 9 or 10 per cent as the result of a rise in the globulin content, despite the fact that the albumin is definitely reduced.

According to Whipple (1914) the normal fibrinogen limits for the human subject may be given as 0.3 to 0.6 per cent with an average of 0.5 per cent per 100 cc. of plasma. (With the Wu method the values range from 0.2 to 0.5 and average about 0.35 per cent (Killian, 1924).) In pneumonia and septicemia fibrinogen is much above normal, reaching 0.9 per cent, while in acute liver injury it drops to a very low level or even to zero in some fatal cases. In chronic liver disease fibrinogen often falls markedly and may cause bleeding (cirrhosis). In general cachexias, such as sarcomatosis, nephritis, miliary tuberculosis, the fibrinogen may be quite low, 0.1 per cent.

NON-PROTEIN NITROGEN. The subject of the non-protein nitrogen has already been made the topic of a special review (Folin, 1922). Reference should be made to this paper for details not permissible in the present discussion.

Although the non-protein nitrogen normally constitutes only about 1 per cent of the total nitrogen of the blood, nevertheless greater interest is attached at the present time to variations in the bodies which form the non-protein than the protein nitrogen. This is due largely to the fact

that the variations in these non-protein constituents give us an insight into some of the processes of anabolism and catabolism. The food nitrogen is carried by the blood to the various tissues and the waste nitrogen to the kidneys, directly or indirectly by the same medium. After a meal containing protein there is a temporary elevation in the non-protein and amino acid nitrogen of the blood. In diseases of the kidney there may be at first only a slight rise in the uric acid or urea, although in the terminal stages of the diseases there is generally a marked elevation in nearly all forms of non-protein nitrogen. The origin and rôle which the various non-protein nitrogenous constituents play in metabolism, as well as the ease of kidney secretion, obviously greatly influence the content of these substances in the blood, both normally and pathologically.

In the estimation of the non-protein nitrogenous constituents of blood it has generally been customary to use whole blood, primarily for the sake of simplicity and economy. Although this scheme suffices for clinical purposes, it is probably well to appreciate that all forms of non-protein nitrogen are not evenly divided between the cells and plasma. Folin and Berglund (1922) have recently given the range in the non-protein nitrogenous constituents of the whole blood, plasma and corpuscles of twelve normal men after a night's fast. From their figures it is evident that the non-protein and urea nitrogen vary within comparatively narrow limits, when the blood is withdrawn in the morning before food has been taken. Urea is an extremely diffusible substance and the slightly greater content found in the plasma would seem to find explanation in the smaller solid content. The content of amino acids and of the undetermined residual nitrogen, in particular, was found to be much (four times) greater in the corpuscles than in the plasma. This difference in the undetermined nitrogen is too great to be explained on the basis of known constituents, such as creatinine, creatine and uric acid. Concerning the possible nature of this residue, Folin and Berglund have ventured the hypothesis that it consists, in part at least, of histons. Jackson (1923), who has recently demonstrated the presence of adenine nucleotide in blood, believes, however, that this nucleotide accounts for a considerable part of the undetermined nitrogen (3 to 5 mgm. nitrogen per 100 cc. in whole blood). It is further of interest that Abel, Pincoffs and Rouiller (1917) were able to show the presence of proteoses in the cellular elements of the blood but not in the plasma. In their study of the non-protein nitrogen of the blood in proteose intoxication Whipple and Van Slyke (1918) found the amino and peptide nitro-

gen about equal. According to Berglund (1922), the creatinine color reacting substance is normally fairly evenly distributed between plasma and corpuscles. The uric acid is likewise divided fairly evenly under normal conditions, although pathologically with high values it may be found chiefly in the plasma. Creatine is, however, present in the cells in approximately three times the concentration found in the plasma.

Although the figures for the amino acid nitrogen are fairly constant after a night's fast, there is always a small increase after each protein meal. The top of the increase in the urea nitrogen after the meal comes five to seven hours later (probable deamination period) and is accompanied by a rapid return of the amino acids to the starting level.

Ammonia was once believed to be present in blood, particularly the portal blood, in appreciable amounts. Folin and Denis (1912 a) demonstrated the fallacy of this view, although their work left the presence of ammonia in the urine unexplained. According to the recent observations of Nash and Benedict (1921), it seems likely that ammonia is not carried to the kidneys for excretion by the blood, but formed in the kidneys from urea in an effort to aid in the excretion of acid. Their findings for the ammonia nitrogen content of blood vary from 0.03 to 0.2 mgm. per 100 cc.

One would hardly expect that the kidneys could eliminate the various nitrogenous waste products with equal readiness. The difference between the ability to excrete uric acid on one hand, and urea and creatinine on the other, is indicated by a comparison of their concentration in normal blood and urine. On this basis the kidneys normally concentrate the creatinine about 100 times, the urea 80 times, but the uric acid only 20 times. Myers, Fine and Lough (1916) have pointed out that as the permeability of the kidneys is lowered in renal disease, this becomes evident in the blood, first by a retention of uric acid, later by that of urea, and lastly by that of creatinine, indicating that creatinine is the most readily eliminated of these three nitrogenous waste products, and uric acid the most difficultly eliminated with urea standing in an intermediate position. Folin (1922) seems scarcely willing to subscribe to this view, pointing to the retention of all three waste products in advanced cases of nitrogen retention. It is quite true that with almost complete or even partial suppression of renal function, as occurs for example in bichloride poisoning, there is the same percentage retention of all three products, but in typical nephritis such cases appear to be the exception rather than the rule.

Non-protein and urea nitrogen. As early as 1902 Strauss made the level of the non-protein nitrogen the basis of a classification of various forms of nephritis, while Widal in 1903 used the blood urea as a prognostic index in advanced nephritis. It was not, however, until ten years later when Folin introduced a simple method of estimating non-protein nitrogen and Marshall described the urease method of urea estimation, that we obtained an adequate appreciation of the normal and pathological variations in the non-protein and urea nitrogen. With further refinements in these methods we now possess very reliable data for these determinations on a variety of conditions. For discussion reference may be made to the papers of Tileston and Comfort (1914) and Berglund (1922).

Normally the non-protein nitrogen of whole blood yields figures between 25 and 35 mgm. per 100 cc. and the urea nitrogen figures between 10 and 15 mgm. Since urea is the chief nitrogenous waste product and its estimation is simpler than that of the non-protein nitrogen, the urea estimation is most often employed. The level of the blood urea is the most reliable single test of renal function at our disposal.

With suppression of renal function the figures for non-protein nitrogen may reach 400 mgm. and urea nitrogen 350 mgm. per 100 cc., the urea nitrogen thus constituting 85 per cent of the nitrogen—the urine ratio. In renal disease the findings range between the above figures and normal. The figures for the urea nitrogen of perfectly normal individuals certainly fall within very narrow limits, when blood is withdrawn after a night's fast. As soon as one passes to hospital patients, however, figures above 15 mgm. are encountered, and figures over 20 mgm. on the usual restricted diet of the hospital are good evidence of impaired renal function. Marked urea retention may occur not only in the terminal stages of chronic nephritis, but also in such conditions as bichloride poisoning, double polycystic kidney, and in some cases of acute nephritis. In nephrosis the findings are comparatively low. Relatively high figures are frequently noted in malignancy, pneumonia, intestinal obstruction, peritonitis, lead poisoning, and sometimes in syphilis and cardiac conditions, due probably to renal complications. In cases of urinary obstruction, such as prostatic obstruction, without complications the urea nitrogen does not generally exceed 20 mgm. In cases of prostatic obstruction the urea is an excellent preoperative prognostic test (Squier and Myers, 1918). It is likewise a helpful prognostic test in the nephritis occurring in early life, since nephritis in children does not so quickly result in urea retention as in the adult.

Since urea is largely of exogenous origin in the blood urea may be subject to considerable variation as a result of dietary influences. Folin, Denis and Seymour (1914) have conclusively shown that lowering the level of protein metabolism serves to reduce the non-protein and urea nitrogen in mild cases of chronic interstitial nephritis. Urea is of less prognostic value than the creatinine in advanced cases of nephritis, but a much better guide to treatment.

It is of interest to note that in eclampsia normal and occasionally subnormal figures for urea nitrogen are found, although the whole blood non-protein nitrogen values are generally slightly high, suggesting an increase in the undetermined nitrogen fraction (Killian and Sherwin, 1921). Employing plasma for the estimation of the non-protein nitrogen, Plass (1924) has obtained normal figures.

Amino acid nitrogen. Van Slyke and Meyer (1912) were the first to supply us with quantitative information regarding the amino acid nitrogen of the blood employing Van Slyke's gasometric method. With this method they confirmed the earlier statements of Folin and Denis (1912), made on the basis of non-protein nitrogen estimations, that after the hydrolysis of proteins to amino acids in digestion, the amino acids are absorbed directly into the blood stream. Folin and Berglund (1922) have considerably extended our information on this point, employing Folin's new colorimetric method, while Greene, Sandiford and Ross (1924) have completed a quite extensive study of the amino acid content of normal and pathologic human bloods with the same method. The latter workers have found that the amino nitrogen content of the blood in a series of 20 normal persons and in more than 400 observations covering 20 pathologic conditions varied between 4.8 and 7.8 mgm. per 100 cc., the average being 6.3 mgm. In such disease conditions as uremia, diabetes, exophthalmic goiter and hepatic insufficiency perfectly normal values were found. It should be noted, however, that the level of amino nitrogen may be increased *a*, by flooding the organism with amino acids arising during digestion; or *b*, by the rapid autolysis of body tissue, as has been observed in cases of leucemia and acute yellow atrophy of the liver.

Uric acid. No advance in our knowledge of the blood uric acid was made from the time of Garrod (1848) until Folin and Denis (1913) reported their first results on the uric acid content of human blood. In a series of unselected cases they found between 1 and 3 mgm. to 100 cc., the average being close to 2 mgm., while in gout their figures varied between 3.5 and 5.5 mgm. Somewhat similar increases were observed

in lead poisoning and leucemia. Practically no elevation of the uric acid was noted in a series of 11 nephritic bloods with only moderate nitrogen retention, but later they reported data (1914) on cases of advanced nephritis in some of which very high values were obtained, up to 10 mgm. These latter observations were confirmed by Myers and Fine (1915), who noted very high figures for uric acid in several cases of terminal interstitial nephritis. In one case the uric acid reached the enormous figure of 27 mgm. shortly before death, while in several cases figures as high as 15 mgm. were observed, values much higher than any noted in gout.

As originally carried out the Folin-Denis method was not entirely accurate, but with the modifications since introduced, the method (especially the recent modification of Benedict, 1922) leaves little to be desired from the standpoint of simplicity and accuracy. The figures which are now regarded as normal for the blood uric acid, however, differ very little from those originally reported by Folin and Denis. Although healthy adults most often yield values between 2 and 3 mgm. per 100 cc. of blood, figures as low as 1 mgm. and as high as 3.8 mgm. may be encountered in strictly normal individuals, the differences probably depending in part upon dietary factors. High blood uric acids must obviously depend upon either an increased formation or a decreased elimination. In leucemia the first factor accounts for the increase, but high uric acids in most other conditions find a probable explanation on the latter basis. Among these may be mentioned gout, nephritis, acute and chronic (but not parenchymatous), eclampsia, cardiac inefficiency, arterial hypertension, lead poisoning, bichloride poisoning, malignancy, acute infections, especially pneumonia and apparently an occasional case of non-gouty arthritis. Kingsbury and Sedgwick (1917) have made the interesting observation that the blood uric acid is high during the first three or four days of life, in harmony with the high uric acid excretion during this period. That starvation raises the level of the blood uric acid has recently been shown by Lennox (1924). In a series of fasting periods lasting eight days or longer, the average increase was from a pre-starvation level of 4 mgm. to a starvation peak of 10.7 mgm. per 100 cc. of whole blood.

It is logical to expect that high values for the blood uric acid would be found in the last stages of chronic nephritis, with the consequent accumulation of all the waste products of nitrogenous metabolism. That the retention of uric acid in nephritis results in a fairly even distribution of this substance in the various body tissues has been shown

by Fine (1915) in tissues obtained at autopsy. The distribution, however, is not quite as uniform as in the case of the urea or even the creatinine, a fact which might be expected from their physical properties.

In 1916 Myers, Fine and Lough called attention to the fact that very high figures for uric acid may be noted, not only in cases of advanced nephritis, but also in the very early stages of the disease, before a retention of either urea or creatinine had taken place. They suggested that when symptoms of gout were absent, a high blood uric acid might serve as an early sign of impaired renal function. Subsequently Baumann, Hansmann, Davis and Stevens (1919) took up a study of this question and concluded "that the uric acid concentration of the blood is a delicate, if not the most delicate, index of renal function at our disposal." Uric acid retention has likewise been noted by Williams (1921 a) in chronic nephritis of moderate degree, although he emphasizes in particular its retention in cardiac decompensation with passive hyperemia of the kidney, where figures for the blood uric acid were found somewhat higher (averages 6.9 and 6.1 mgm. per 100 cc.). Krauss (1922) likewise regards the retention of uric acid the first sign of impaired kidney function. Williams (1921 b) has further observed uric acid retention unassociated with urea retention in eclampsia, as have Killian and Sherwin (1921) independently. It is of interest regarding the uric acid in nephritis that early in the disease the values observed may be somewhat higher (7 to 9 mgm.) than at a later stage (5 to 6 mgm.), due possibly to dietary restrictions, although during the last days of life the amount may be markedly increased (10 to 20 mgm.). Czoniczer (1922) believes that these high uric acid values fluctuate with the uremic symptoms.

Owing to the fact that the tophi found in gout have long been recognized to contain deposits of sodium urate, the uric acid of the blood possesses a special interest in this condition. Following the investigations of Folin and Denis (1913), a number of different workers took up a study of this question, among whom may be mentioned Pratt (1913, 1916) and Fine (1916). Pratt (1922) has recently reviewed the subject of gout.

Typical cases of gout generally show comparatively high figures for the blood uric acid, although the figures are not as high as those which may be encountered in chronic interstitial nephritis. Furthermore, the uric acid tends to remain high in gout in spite of dietetic measures. From normal figures of 2 to 3 mgm. per 100 cc. of blood the uric acid may increase to as much as 4 to 9 mgm. in gout, but it does not follow that

these uric acid accumulations are infallible signs of gout since, as noted above, similar uric acid figures may be found in nephritis. Although the blood content of uric acid may be of considerable value in the diagnosis of gouty arthritis, it should be borne in mind that cases of infectious arthritis occasionally show an increase in the uric acid. Here the retention of uric acid, in contrast to gout, is generally associated with an increased urea and non-protein nitrogen. Such cases of arthritis are the exception rather than the rule.

It has been recognized for some time that the administration of salicylates and phenylcinchoninic acid and certain of their derivatives results in an increased excretion of uric acid in the urine. Folin and Lyman (1913) and Fine and Chace (1914) have shown that the administration of phenylcinchoninic acid is accompanied by a marked drop in the uric acid content of the blood, and later the same was shown to be true of salicylates by Fine and Chace (1915) and Denis (1915). As a result of these studies on the blood it became clearly evident that the increased elimination of uric acid after the administration of these drugs was due to increased renal function. Although this action is most marked in the case of the uric acid it is by no means specific, and Myers and Killian (1921), in selected cases with moderate nitrogen retention, have been able to demonstrate a reduction not only in the uric acid, but also in the urea and in one instance in the creatinine. It should be noted, however, that in the terminal stages of chronic interstitial nephritis Fine and Chace (1915 a) have shown that phenylcinchoninic acid has almost no influence on the excretion of uric acid, indicating that the renal cells can no longer be stimulated to increased activity.

Creatinine. Practically no quantitative information on the creatinine of the blood was available prior to 1914 when Folin and Denis reported observations on a series of normal and miscellaneous cases and nine cases of uremia.

For perfectly normal individuals the creatinine of the blood amounts to 1 to 2 mgm. per 100 cc., the findings for the strictly normal being nearer 1 than 2 mgm. As soon as one passes to hospital patients, however, higher values are found. Although the great majority of cases without renal involvement show creatinine figures on the whole blood below 2.5 mgm. per 100 cc., occasionally figures as high as 3.5 mgm. are encountered that are not readily explained. It may be noted, however, that apparently a slight retention of creatinine (figures between 3 and 4 mgm.) occurs in syphilis, certain heart conditions, sometimes in fevers, and in some cases of advanced diabetes. Creatinine figures above 3.5

mgm. are almost invariably accompanied by an appreciable urea retention and this is generally true of those above 3 mgm. Many of the cases below 4 mgm. show improvement, but with over 4 mgm. the reverse is the case. It would appear from this that an appreciable retention of creatinine, i.e., over 4 mgm., does not occur until the activity of the kidneys is greatly impaired. That such should be the case is quite natural to expect, since creatinine is normally the most readily eliminated of the three nitrogenous waste products, uric acid, urea and creatinine.

In a recent communication Behre and Benedict (1922) have presented evidence which indicates that creatinine as such does not exist in normal blood, although their data are not quite as definite in the case of nephritic bloods. How then is the presence of creatinine in the urine to be explained? Behre and Benedict suggest that the kidney may be able to concentrate the creatinine from extremely dilute solutions or produce the creatinine from some common precursor substance in the blood. They are inclined to the latter view and suggest creatine as the probable precursor. They have presented real evidence of the existence of creatine in blood, but creatine has not ordinarily been looked upon as a waste product, as would be called for in this hypothesis. Furthermore, this view is not in harmony with what we know regarding the transformation of creatine to creatinine. These observations of Behre and Benedict may be reconciled with our present views by supposing that the chromogenic substance present in blood is closely related to creatinine and readily converted to creatinine in the kidney. It seems difficult to explain the increase in the chromogenic substance in nephritic bloods on any other ground than impaired excretion of creatinine. It is of interest in this connection that the taking of creatinine by mouth leads to an increase in the creatinine reaction of the blood (Berglund, 1922). Furthermore, the increase in the blood creatinine in chronic nephritis goes hand in hand with a decreased renal excretion (Myers and Fine, 1915).

In their studies on nitrogen retention Myers and his co-workers soon noted that the creatinine of the blood was appreciably increased only after considerable retention of urea had already taken place and the nephritis was rather far advanced (Myers and Lough, 1915; Myers and Killian, 1919). It was further observed that those cases in which the creatinine had risen above 5 mgm. per 100 cc. of blood rarely showed any marked improvement, and almost invariably died within a comparatively limited time. The only exceptions were cases where the retention was due to some acute renal condition, such as mild bichlo-

ride poisoning, acute nephritis and intestinal obstruction. The prognostic value of the blood creatinine appears to have received quite general confirmation (Rosenberg, 1916; Höst and Hatlehol, 1921–22; Williams, 1921; Rabinowitch, 1921; Rowntree, 1922).

Theoretically, the amount of the increase of the blood creatinine should be a safer index of the decrease in the permeability of the kidney than the urea, for the reason that creatinine on a meat-free diet is entirely endogenous in origin and its formation (and elimination normally) very constant. Urea, on the other hand, is largely exogenous under normal conditions and its formation consequently subject to much greater fluctuation. For this reason a lowered nitrogen intake may reduce the work of the kidney in eliminating urea, but cannot affect the creatinine to any extent. Apparently the kidney is never able to overcome the handicap of a high creatinine accumulation.

Creatine. Normally the creatine content of the blood amounts to 3 to 7 mgm. per 100 cc., although the amount may be greatly increased in the last stages of nephritis along with other non-protein nitrogenous substances. Creatine appears to be present in the cells in much greater concentration than in the plasma, as might be expected from its content in other body tissues. Its estimation in the blood has not been shown to possess any great practical importance, although as just indicated it is considerably increased in the terminal stages of nephritis. The relationship of the creatine of the plasma to the excretion of creatine in the urine is of some interest. As shown by Berglund (1922), the taking of pure creatine by mouth in doses of 4 grams is sooner or later followed by an increase in the creatine figures of the plasma and the smallest appreciable increase in the creatine of the plasma is accompanied by the excretion of creatine in the urine. Hunter and Campbell (1918) were the first to point out this relation between the creatine of the plasma and creatinuria.

Sugar. Although we have long possessed sufficient data on the sugar of the blood to give us a clear insight into the variations which occur in disease (see Bang, 1913), it was not until simple micro methods of blood sugar estimation had been devised by Bang (1913a) and Lewis and Benedict (1913) that this determination became a common clinical procedure. There are now available a number of simple and reasonably accurate micro methods of blood sugar estimation in addition to those of Bang and Benedict. Among these should be mentioned the methods of Folin and Wu (1920) and Hagedorn and Jensen (1923). Stimulated by these new methods many investigations have been carried out deal-

ing with the sugar of the blood. It was the topic of a review by Macleod in 1921.

The blood sugar of the normal human subject appears to fall somewhere between 0.09 and 0.12 per cent, the average being about 0.10 per cent. Depending upon the method which is employed for the estimation, one may obtain figures differing by as much as 0.02 per cent in normal blood, while with pathological bloods the differences, as shown by Höst and Hatlehol (1920), may be somewhat greater. Slightly higher figures appear to be obtained by the picric acid method of Benedict in its various modifications than by most of the other methods. That the reducing power of the blood is due in large part to glucose seems certain, although the various methods appear to be influenced by other reducing substances. The question of the actual sugar content of glucose in normal blood is one of great theoretical interest and importance. The figures obtained by the various methods, however, differ so little relative to the variations which occur in disease that the question of the method scarcely enters into a discussion of the blood sugar findings in disease.

The figure of 0.10 per cent for normal individuals given above applies to observations made in the morning previous to the intake of any carbohydrate. After a meal rich in carbohydrate there may be an appreciable rise in the sugar content of the blood, 0.12 to 0.14 per cent, while after the intake of even moderately large amounts of glucose, the hyperglycemia, 0.15 to 0.16 per cent, may be sufficient to induce a slight temporary glycosuria (glycuresis). The great majority of hospital cases show practically normal figures for blood sugar, although occasionally figures of 0.12 to 0.15 per cent are encountered that are not readily explained.

The above statements apply to venous blood, although after a night's fast there appears to be little difference between venous and arterial blood. Foster (1923) has recently shown that finger blood is virtually arterial blood, and that after the ingestion of glucose (or starch) the sugar content of finger blood is materially higher than venous blood.

Conditions of hyperglycemia are much more common and of greater clinical interest than those of hypoglycemia, owing primarily to the fact that diabetes belongs to the former group. Among other conditions which frequently show a moderate hyperglycemia are nephritis and hyperthyroidism. Hyperglycemias are also found in pancreatic disease, in some cases of cholecystitis and occasionally in infections. Hypo-endocrine function (pancreas excepted) would appear to result

in hypoglycemia, and comparatively low blood sugars have been observed in myxedema, cretinism, Addison's disease, pituitary disease and other less clearly defined endocrine disorders, such as muscular distrophy.

All forms of glycosuria are accompanied by hyperglycemia, if we except the glycosuria produced by such substances as phlorhizin and uranium, and the analogous clinical condition, renal diabetes. The normal *threshold* of sugar excretion (i.e., the point of glycuresis) is about 0.17 to 0.18 per cent (Hamman and Hirschman, 1917). With blood sugar concentrations of 0.15 to 0.20 per cent the appearance of sugar in the urine is apparently dependent on whether or not diuresis exists, glycosuria appearing especially in the latter case. When the threshold has been passed, however, the overflow of sugar into the urine may continue until the concentration in the blood has fallen nearly to normal. Mild cases of diabetes usually have a normal threshold, although some severe cases apparently have a lowered threshold, increasing the severity of the condition. Ordinarily in the early stages of the disease there is a fairly direct relationship between the hyperglycemia and glycosuria. In the latter stages of the disease, however, cases are frequently encountered with marked hyperglycemia and only slight glycosuria, showing that the threshold has been raised (Myers and Bailey, 1916; Hamman and Hirschman, 1917; Williams and Humphreys, 1919). Myers and Bailey found a fairly direct relation between the height of the sugar threshold and nephritic symptoms, while Fitz (1917) has shown that impaired renal function exists in many cases of advanced diabetes. Hamman and Hirschman state that in the terminal stages of diabetes a high threshold is almost the rule, but emphasize that the renal impermeability is by no means constantly associated with a nephritis that is demonstrable clinically. As shown by Hamman and Hirschman and Bailey (1919), the sugar threshold is often above 0.2 per cent (and may reach 0.3 per cent) in chronic nephritis. Some cases of nephrosis, however, may show a low grade glycosuria independent of the blood sugar concentration. In fact, they may show many of the phenomena of renal diabetes. In so-called "renal diabetes" the cause of the glycosuria is obviously due to a threshold point below the level of the normal blood sugar.

In mild cases of diabetes the hyperglycemia is not excessive, generally 0.2 to 0.3 per cent, but in severe cases figures up to and even above 1.0 per cent have been reported (Myers and Bailey, 1916). Since the glycosuria of diabetes is dependent upon hyperglycemia, it is apparent

that the hyperglycemia is more fundamental, the glycosuria apparently being only a safety factor. In the early stages of the disease the glycosuria serves as a fairly reliable index of the hyperglycemia, but when the condition has become chronic, the sugar threshold, as already pointed out, is generally raised, and the glycosuria consequently serves as a poor guide to the glycemia. The general use of the blood sugar determination as a guide to diabetic treatment has been slow for the reason that many clinicians were content simply to employ the older and simpler methods of urine examination. It was ultimately recognized, however, that a hyperglycemia might still be present despite the fact that the urine was sugar-free. (As is now well known, a normal twenty-four hour specimen of adult urine contains on the average about 0.8 gram sugar.) With the appreciation of this fact the question of the blood sugar level to be maintained in the treatment of diabetes became a much controverted question. Some believed that the blood sugar should be maintained at the normal level even though this necessitated a very rigid adherence to the Allen starvation treatment, while others believed that a more liberal diet was permissible. In this connection Williams (1919) has stated: "Our experience has led us to the conclusion that it is desirable to maintain the blood sugar level as nearly normal as possible even though severe restrictions in diet may be necessary for the purpose, notwithstanding the fact that in most cases the high threshold will permit of a much more liberal diet without the appearance of sugar in the urine." On the other hand, Mosenthal (1918) has suggested that the raised threshold may be merely a protective measure to adjust carbohydrate metabolism for the more advantageous utilization of glucose.

It was at this stage in the development of our knowledge of diabetes that Banting and Best (1922) made their epoch-making discovery of the pancreatic hormone, insulin. (Insulin has just been reviewed by Macleod (1924), in whose laboratory Banting and Best carried out their experiments.) Had this discovery been made ten years earlier, its clinical application would have been much more difficult, for the reason that we then possessed no simple method of blood sugar estimation. Blood sugar has served as the basis for the standardization of the insulin unit, and likewise as a guide to its therapeutic administration. The unit of insulin originally adopted was the amount required to lower the normal blood sugar of a one kilogram rabbit to 0.045 per cent, at which point convulsions generally occur. A unit of insulin, when injected (subcutaneously) into a diabetic will enable him to utilize from 1 to 4 grams additional carbohydrate depending upon the severity of the

diabetes and the number of units given. The height of the blood sugar constitutes the most reliable guide to the administration of insulin. Insulin apparently enables a diabetic to utilize carbohydrate in a normal manner. Briggs, Koechig, Doisy and Weber (1924) have recently shown that insulin causes a decrease in the concentration of glucose, inorganic phosphate, and potassium with a parallel increase in lactic acid in the blood of normal animals.

An idea of the action of insulin in diabetes may be obtained from the illustrative case (unpublished analyses of Killian) given in table 3.

TABLE 3

Some blood and urine findings after insulin, on A. Y., aged 20 years. 21 units of "Iletin" given at 10:10 a.m

TIME 1/10/23	BLOOD ANALYSES		TIME	URINE ANALYSES			
	Sugar	CO_2 combining power		Volume	Sugar	Sugar	Total acetone bodies
	per cent	*cc. to 100*		*cc.*	*per cent*	*gm.*	*gm.*
10:00	0.302	37	10:20	210	3.5	7.35	0.76
11:10	0.274	40	11:20	65	3.0	1.95	0.29
12:10	0.220	53	12:20	30	1.5	0.45	0.10
			1:20	305	0.01	0.03	0.06
2:10	0.184	57	2:20	255	0.01	0.02	0.03
3:15	0.193	52	3:20	45	0.11	0.04	0.08
4:15	0.157	51	4:20	115	0.08	0.09	0.06
5:10	0.190	51	5:20	35	0.19	0.09	0.10

As will be noted the influence upon the urine sugar is more rapid and much more striking than in the case of the blood sugar. Obviously the sugar threshold is an important factor in the disappearance of sugar from the urine, the threshold in this case being apparently above 0.2 per cent. Whereas the lowest urine sugar was reached at the end of three to four hours, the lowest blood sugar, 0.157 per cent, was not obtained until the sixth hour. After the blood sugar had dropped below the threshold point, and as noted above this is often raised in diabetes, the urine furnished no further information. Chiefly for this reason the blood sugar is a much more reliable guide for the insulin treatment, despite the striking influence upon the urine sugar; and it is better that at the lowest point there should be a slight hyperglycemia, as in this case, than that the dangers of a marked hypoglycemia should be encountered.

It has been quite generally recognized that hyperglycemia may occur in nephritis, although in this condition the blood sugar rarely exceeds

0.2 per cent (Hopkins, 1915; Myers and Bailey, 1916; Hamman and Hirschman, 1917; Williams and Humphreys, 1919). Different methods of blood sugar estimation do not always give concordant results in nephritis, and some have disputed the presence of a hyperglycemia. Hamman and Hirschman have studied the sugar tolerance in nephritis and state that in many cases there is a profound change in the carbohydrate metabolism, the blood sugar curve after the ingestion of glucose resembling the "diabetic curve."

That cases of hyperthyroidism frequently show high blood sugars (although not often in the fasting state), and that alimentary glycosuria may be readily provoked in this condition has been recognized for some time (Hamman and Hirschman, 1917; Denis, Aub and Minot, 1917; Janney and Isaacson, 1918). Blood sugar observations obtained after glucose tolerance tests have given us much more definite information regarding the carbohydrate tolerance of individuals with endocrine disorders than the older studies made on the urine, chiefly for the reason that the threshold point of renal excretion is not here a factor in the test. Experimental proof that hypoglycemia results from hypo-endocrine function was obtained by Janney and Isaacson in the case of the thyroid, where hypoglycemia regularly developed after thyroidectomy. Low blood sugar values, figures ranging from 0.06 to 0.09 per cent, have been reported in myxedema, cretinism, Addison's disease, pituitary disease and other less clearly defined conditions such as muscular dystrophy. The last mentioned condition has been discussed by Janney, Goodhart and Isaacson (1918) and McCrudden and Sargent (1918). In pronounced hypo-endocrine conditions there is very little rise, if any, in the blood sugar after the ingestion of the glucose (1.75 gram per kilo of body weight) employed for the carbohydrate tolerance test, but in hyperthyroidism the blood sugar one hour after taking the glucose generally shows a blood sugar of 0.2 per cent or more, in contrast to the normal rise to 0.15 per cent.

Blood lipoids (*fat, lecithin and cholesterol*). In a study of the blood lipoids during fat assimilation, Bloor (1916a) has observed that *1*, the total fatty acids increase in both plasma and corpuscles but the increase is generally more marked in the corpuscles; *2*, lecithin increases greatly in the corpuscles, but only slightly in the plasma; *3*, no definite change takes place in the quantity of cholesterol, and *4*, a fairly constant relationship exists between the total fatty acids and lecithin of the whole blood and corpuscles. From this Bloor suggests: *a*, that the blood corpuscles take up the fat from the plasma and transform it into lecithin;

b, that most, if not all, of the absorbed fat is so transformed; and therefore *c*, that lecithin is an intermediary step in the metabolism of the fats.

In normal bloods the lecithin content of the corpuscles is approximately double that of the plasma, while the cholesterol and total fatty acid values are almost always lower in the corpuscles than in the plasma. The value for lecithin in the corpuscles is generally about twice that of the cholesterol, while in the plasma their values are nearly equal. According to Bloor, the ratio between these constituents is quite constant in normal blood (especially plasma) and remains so in most of the pathological samples, suggesting a definite relationship between these constituents, and making it probable that cholesterol (in the form of its esters) has a part in fat metabolism.

The most characteristic feature of pathological conditions is the increase of total fatty acids and fat both in plasma and corpuscles, and the decrease of lecithin in the plasma. Since the fat is probably to be regarded as the inactive form of the body lipoids, the form in which they are stored, and the lecithin as the first step in the utilization, an undue accumulation of fat or a notably decreased value for lecithin, probably indicates a diminished activity of the fat metabolism.

In severe diabetes (Bloor, 1916b) the blood lipoids are all greatly increased but the ratios between these constituents are practically normal. The fact that the cholesterol increases parallel with the fat in diabetic blood, even in severe lipemia, supports the view that probably cholesterol plays an important rôle in fat metabolism. Since cholesterol may be rather simply estimated it affords a practical method of gauging the severity of diabetic lipemia. In mild diabetes the blood lipoids may be practically normal.

While there is no certain evidence that the abnormalities in the blood lipoids are responsible for anemia, the low values for cholesterol, which is an antihemolytic substance, and the high fat fraction, which may indicate the presence of abnormal amounts of hemolytic lipoids in the blood, are possible causative factors.

According to Bloor (1917), the changes in the blood lipoids in severe nephritis are a high fat in the plasma and corpuscles and high lecithin in the corpuscles. These abnormalities are the same as are found in alimentary lipemia and may be regarded as the result of a retarded assimilation of fat in blood, due possibly to metabolic disturbances. As pointed out by Epstein (1922), a marked lipemia and hypercholesterolemia are found in nephrosis.

Normally the "*total fat*" content of the blood plasma amounts to 0.6 to 0.7 per cent, but in severe diabetes figures as high as 26 per cent have been observed. In diabetic cases of ordinary severity, however, the figures amount to about 1.5 per cent. Nephritics frequently show a moderately increased fat although the figures (nephrosis excepted) rarely reach 1 per cent.

The normal figures for *lecithin* may be given in round numbers as 0.2 per cent for plasma, 0.3 per cent for whole blood and 0.4 per cent for corpuscles. In diabetes there is an increase in the lecithin of both the corpuscles and the plasma, although in severe lipemia it is more noticeable in the latter. In anemia the lecithin of the plasma in particular is lowered, while in nephritis there is a noteworthy increase in the corpuscles.

Cholesterol occurs in the blood in both the free and combined state. Free cholesterol is present in the corpuscles and to some extent in the plasma, while the cholesterol esters are present in the plasma alone. Bloor and Knudson (1917) have found that in whole blood the average percentage of cholesterol in combination as esters is about 33.5 per cent, and in the plasma 58 per cent of the total cholesterol. Normally, the concentration of cholesterol is nearly the same in the plasma (0.15 to 0.18 per cent) and whole blood (0.14 to 0.17 per cent) although, if anything, the plasma content is slightly higher, but pathologically it is subject to much greater variations owing to the fact that the cholesterol content of the corpuscles remains fairly constant. In general it may be stated that hypercholesterolemia is found in arteriosclerosis, nephritis, nephrosis, diabetes (especially with acidosis), obstructive jaundice, in many cases of cholelithiasis, in certain skin diseases, in the early stages of malignant tumors, and in pregnancy. The chief condition in which low values for cholesterol are found is anemia. We have encountered cholesterol values for whole blood above 1.0 per cent in diabetes, 0.25 to 0.83 per cent in nephrosis and as low as 0.07 per cent in pernicious anemia (Myers, 1924).

As pointed out above, cholesterol constitutes an excellent index of the degree of lipemia in diabetes, while the decrease in this antihemolytic substance in the plasma in anemia would appear to be of considerable significance. The one disorder, however, in which cholesterol appears to be diagnostic is nephrosis. We owe especially to Epstein (1922) recognition of the fact that typical nephrosis is associated with marked hypercholesterolemia.

ACETONE BODIES. Owing to the importance which the acetone bodies hold in the acidosis, or more specifically the ketosis, occurring particularly in diabetes, the quantities of these substances—*acetone*, *aceto-acetic acid* and *β-hydroxybutyric acid*—present in normal and pathological human blood is of considerable interest. Methods have been described by Marriott (1913–14), Van Slyke and Fitz (1917) and Hubbard (1921) for their estimation in blood, the method of Van Slyke and Fitz being especially suited to large amounts of acetone bodies, while that of Hubbard is suited to small amounts. Acetone is a very diffusible substance and appears to be readily and rapidly eliminated by the kidneys. For this reason the concentration in the urine is considerably greater than in the blood, and likewise probably more significant. The amount of the β-hydroxybutyric acid present in both blood and urine is ordinarily in excess of the combined acetone-acetoacetic acid fraction, often exceeding the latter by two or three times. although according to the observations of Hubbard this is by no means an invariable rule. Hubbard considers the values for normal bloods to range from 0.1 to 1.0 mgm. per 100 cc. for acetone from all three acetone bodies. According to Van Slyke and Fitz the total acetone bodies of the blood normally amount to 1.3 to 2.6 mgm. per 100 cc., calculated as acetone. In diabetes the figures range from normal findings to as high as 250 mgm. Cases under ordinary good control, however, show figures under 10 mgm. Killian (1923) has observed that following the administration of insulin to diabetics the rise in the blood bicarbonate parallels the decreased elimination of acetone bodies in the urine and the decreased blood content. The highest blood bicarbonate is observed simultaneously with the lowest urine content of acetone bodies (see table 3). Moore (1916) has studied the acetone bodies of the blood in children using the older Marriott method, the figures obtained for normal children being considerably higher than those given above for adults. In general he found the values increased in acute febrile conditions, while in a case of recurrent vomiting a figure as high as 170 mgm. was observed.

MINERAL CONSTITUENTS. In comparison with the organic constituents of the blood the mineral constituents undergo comparatively small changes. The values for the sodium, potassium, calcium and magnesium of the serum remain remarkably constant in health, while in the case of the potassium and magnesium no significant variations have been encountered in disease. Pathological variations in the sodium sometimes occur, along with somewhat similar variations in

the chlorine, while low values for calcium are found notably in parathyroid deficiency and terminal nephritis. The pathological variations in the inorganic phosphorus are quite wide. Until less than ten years ago our knowledge of these constituents was derived largely from analyses published many years ago by C. Schmidt (1850), Bunge (1876) and Abderhalden (1898). Although many workers have recently devoted attention to this field the contributions which have emanated from Howland's laboratory have been especially noteworthy.

An idea of the normal range of these various mineral constituents in human blood, the average findings and the limits of pathological variation may be obtained from table 4 below.

TABLE 4

Mineral constituents of blood

CONSTITUENT	NORMAL		PATHOLOGICAL
	Range	Average	Range
	mgm. to 100 cc.	*mgm. to 100 cc.*	*mgm. to 100 cc.*
Sodium (serum) as Na	320–350	335	220–460
Potassium (serum) as K	18–22	20	10–35
Potassium (whole blood) as K	150–250	200	50–400
Calcium (serum) as Ca	9–11	10	2–12
Magnesium (serum) as Mg	15–3	2.5	1–4
Iron (whole blood) as Fe		50	15–70
Chlorides (plasma) as Cl	340–370	360	300–510
Chlorides (plasma) as NaCl	570–620	600	500–850
Chlorides (whole blood) as NaCl	450–520	480	120–700
Phosphorus as P			
Inorganic (serum)	2.5–5.5	3.5	1–40
Lipoid (plasma)	5–12	7.5	
Organic (corpuscles)	40–75	53	
Sulfates as S	0.5–0.9	0.5	0.4–16

Sodium. It has been recognized for many years that sodium is found chiefly in the body fluids, while potassium is a constituent principally of the cellular tissue. As might be expected, therefore, sodium is found in the plasma, and potassium chiefly in the corpuscles. (In the dog and cat sodium, instead of potassium, is present in the corpuscles, but in man sodium appears to be entirely absent.) We have long known the sodium content of human blood serum from the observations of Schmidt (1850) and Wanach (1888), but until the advent of the Kramer-Tisdall method (1921a), the figures available were too limited

to permit generalizations. Kramer and Tisdall (1922) have shown that sodium is practically absent from the corpuscles, and that normally the serum content is very constant, close to 335 mgm. per 100 cc. The normal range of variation appears to fall between 320 and 350 mgm., but figures as low as 220 mgm. have been observed by Kramer and Tisdall (1921a) in severe acidosis in children, while Denis and Hobson (1923) have encountered figures as high as 460 mgm. in nephritis. The highest figures for Na (and Cl) were observed in cases with comparatively little nitrogen retention. The sodium figures recently obtained by Salvesen and Linder (1923) in a study of the mineral constituents of the serum in Bright's disease and heart failure were all essentially within normal limits.

Potassium. Although the information available at present concerning the potassium content of blood is somewhat limited, considerably more is known than in the case of sodium. (Some years ago Abderhalden (1898) reported analyses of the blood of different animals. The figures obtained for potassium are of considerable interest. In the dog and cat practically identical figures were found for the serum and whole blood. This amounted to about 22 mgm. per 100 cc., almost the exact amount found in the serum of the various animals examined. In the ox, sheep and goat the figures for whole blood were about one and one-half that of the serum, while in the horse, pig and rabbit the potassium concentration of the whole blood was about ten times that of the serum.) The potassium content of human blood has recently been considered by Macallum (1917), Kramer and Tisdall (1921), Myers and Short (1921), Denis and Hobson (1923) and Salvesen and Linder (1923), who are in close agreement that the potassium of normal human blood serum or plasma is a relatively constant quantity and amounts to close to 20 mgm. K per 100 cc. In strictly normal individuals the potassium content of the serum apparently rarely falls outside the limits of 18 to 22 mgm., although in some pathological conditions such as nephritis, which may be accompanied by some changes in the other cations and anions, somewhat wider variations may be found, 10 to 35 mgm. Wilkins and Kramer (1923) examined the serum in a variety of conditions and found the potassium to fall within 18 to 22, except in certain cases of nephritis and tetany where figures from 23 to 29 mgm. were found. It has been suggested by Smillie (1915) that uremic symptoms may be due to potassium poisoning. The observations so far recorded, however, do not support the idea that the potassium of the serum ever reaches a sufficiently high level to be responsible for the clinical symptoms observed.

It is of interest from the standpoint of intermediary carbohydrate metabolism that Briggs, Koechig, Doisy and Weber (1924) have recently shown that when insulin is administered to normal animals (dogs) there is a simultaneous decrease in the concentration of glucose, inorganic phosphate and potassium, with a corresponding rise in lactic acid. The average drop in 11 experiments was, in the case of inorganic P, from 3.0 to 1.3 mgm. and in the case of K from 19.2 to 14.6 mgm. per 100 cc.

The potassium content of whole blood depends in large measure upon the cell content, and appears to vary somewhere between 150 and 250 mgm. per 100 cc. in the normal human subject. In primary and secondary anemia the amount may obviously be very low. Pathologically the potassium content of the serum or plasma is of greater interest.

Calcium. According to the recent observations of Kramer and Tisdall (1922), human blood corpuscles do not appear to contain appreciable amounts of calcium. This being the case, significant changes in the blood calcium are best shown, as pointed out by Halverson, Mohler and Bergeim (1917), by analyses made upon the serum or plasma. The calcium content of human blood serum is normally very constant at somewhere between 9 and 11 mgm. per 100 cc., the figures averaging slightly higher in children than adults.

Fifteen years ago W. G. Macallum and Voegtlin (1909) recognized the reduction in the calcium content of the blood following the removal of the parathyroids in animals and the development of tetany. The symptoms of tetany were found to be relieved by the administration of calcium salts. In 1917 Howland and Marriott observed that the calcium content of the serum is greatly reduced in infantile tetany, figures between 3.5 and 7.0 mgm. being found where symptoms of tetany were present. These observations were confirmed by Denis and Talbot (1921) and others. The tetany is relieved only by the persistent and constant administration of calcium, but with serum calcium figures of 7.5 mgm. or more symptoms do not appear. There appears to be some definite relation between the increased irritability of the neuromuscular mechanism, which is the essential phenomenon in infantile tetany, and the reduction in the calcium of the blood. Salvesen (1923) has recently shown that when parathyroidectomized dogs are given calcium chloride intravenously, raising their serum calcium from about 5 to 15 mgm., the calcium drops back to the initial level within twenty-four hours. So long, however, as the dogs are kept on a milk diet, thus receiving calcium constantly, they remain free from symptoms of tetany. About 60 to 70 per cent of the total serum calcium is in the diffusible or

ionized form (von Meysenbug, Pappenheimer, Zucker and Murray, 1921). According to the observations of Salvesen and Linder (1923 a) it is this fraction of the serum calcium which is reduced after parathyroidectomy, as the serum proteins remain normal.

Marriott and Howland (1916) were the first to point out that in advanced nephritis with phosphate retention and acidosis the serum calcium is markedly reduced, figures as low as 2 to 4 mgm. This observation has been confirmed by a number of workers (Halverson, Mohler and Bergeim, 1917; Feigel, 1920; Denis and Hobson, 1923; Salvesen and Linder, 1923; Schmitz, Rohdenburg and Myers, 1924), although the figures reported have only occasionally been below 6 mgm. per 100 cc. of serum. Salvesen and Linder have recently pointed out that the decrease in serum calcium in non-uremic cases of Bright's disease without phosphate retention parallels the decrease in plasma protein. They believe that this is due to a decrease in the non-diffusible portion which is bound to the plasma proteins. In cases with phosphate retention this is probably the cause of the reduction in the calcium. This explanation has been suggested by the experiments of Binger (1917).

Magnesium. The normal magnesium content of the blood of both adults and children (as Mg.) generally falls between 2 and 3 mgm. per 100 cc. of plasma or serum, although with pathological bloods a somewhat wider range of 1 to 4 mgm. is found. A considerable number of different pathological conditions has been studied, but the findings differ very little from those found during health and do not appear to be characteristic of any special pathological condition.

Iron. Iron is present in hemoglobin to the extent of almost exactly one-third of one per cent, which would make the content of normal human blood about 50 mgm. per 100 cc., calculated as Fe. Pathologically, it varies directly with the hemoglobin content. Iron does not appear to be present normally in the plasma.

Chlorides. Although accurate data on the blood chlorides have long been available, and furthermore large numbers of chloride estimations have been made with recent micro methods, it is possible to make but few generalizations regarding pathological variations in the blood chlorides.

Some of the observations recorded in the literature give the chloride content of whole blood, others the content of the plasma or serum. Normally the chloride content of whole blood as NaCl amounts in round numbers to 0.45 to 0.50 per cent, while for the plasma the figures are about 0.12 per cent higher, i.e., 0.57 to 0.62 per cent. Since the

plasma, rather than the whole blood, bathes the tissues of the body, it would seem more logical to study the chloride content of the plasma. Unfortunately, unless the plasma is quickly separated from the corpuscles there appears to be a gradual change (increase) in its chloride content, owing to the passage of carbon dioxide from the plasma into the corpuscles (or its escape into the air) and of Cl ion from the corpuscles into the plasma (Van Slyke and Cullen, 1917). For this reason unless the plasma can be separated from the cells rather quickly after the blood is drawn, whole blood analyses are more trustworthy.

Obviously the cell volume is here a factor to be considered, since the cells contain only about half as much chlorides as the plasma. There appears to be a fairly direct relation between the hemoglobin content and cell volume. According to Norgaard and Gram (1921) the plasma chloride is ordinarily constant at 0.61 per cent, likewise the cells at 0.31 per cent, the only serious divergence from this latter figure being found in pernicious anemia, where the average content is calculated to be 0.23 per cent. They believe that unless the factor of cell volume is taken into account (they supply a formula), the blood chlorides furnish little more information than a simple cell volume determination.

Schmidt (1850) in his classic studies on the blood with special reference to cholera, gives figures for the chloride content of whole blood and plasma. Low figures were obtained in many cases of cholera, while in a case of "chronic edema with albuminuria" a definite increase was observed.

McLean (1915) has devoted considerable attention to the subject of blood chlorides. In a fairly large series of normal individuals he found the plasma chloride to vary from 0.57 to 0.62 per cent with a very constant chloride threshold of about 0.562 per cent. The threshold was calculated from the formula of Ambard and Weill and confirms their observation on the point. McLean considered the question of the plasma chlorides in a number of pathological conditions, the lowest observation being 0.50 per cent in a diabetic and the highest 0.84 per cent in a cardionephritic shortly before death. In general, relatively increased concentrations of chlorides were found in the plasma in certain forms of cardiac and renal disease, while decreased concentrations were noted in certain diabetic and fever patients, also after the action of digitalis, the decreased concentration apparently resulting from a lowering of the chloride threshold. Failure to excrete chlorides in pneumonia was found to be associated with a lowered concentration of chlorides in the plasma, excretion reappearing with a rise in the plasma chloride.

Edema was found to be accompanied by a relatively increased concentration of chlorides in the plasma, which ordinarily returned to the normal state with the disappearance of the edema.

In 1920 Allen published a paper on arterial hypertension in which he endeavored to show that salt retention (as shown by the plasma chloride) is the cause of pure hypertension, and also of hypertension in many cases of kidney disease. With Sherrill (1922) he has submitted added data. Mosenthal and Short (1923) and O'Hare and Walker (1923) have not, however, been able to confirm these findings.

The most striking fall in blood chlorides has been observed in experimental pyloric obstruction in dogs (Hastings, Murray and Murray, 1921; Hayden and Orr, 1923). This is accompanied by a proportionate rise in the blood bicarbonate. Hastings has shown that the drop in chloride concentration occurs in both plasma and cells. According to Hayden and Orr, the fall in chlorides is not due to loss of chlorides in gastric juice, but probably to the taking up of the chlorine somewhere in the process of protein destruction. It is of interest that Killian (1921) has observed similar low figures for the blood chlorides in bichloride poisoning. Hayden and Orr (1923a) have employed sodium chloride therapeutically in pyloric and intestinal obstruction and believe that it has a marked effect in preventing and controlling the toxemia.

In general it may be stated that high blood chlorides have been found in nephritis, certain cardiac conditions, in eclampsia, prostatic obstruction, in anemia and some cases of malignancy, while low values have been observed notably in fevers, diabetes and pneumonia. It is of interest that in nephrosis, despite the marked edema, there is little or no salt retention, if one may judge from the whole blood chlorides (Myers, 1924). This is quite contrary to what might be anticipated. The function of excreting chlorides in interstitial nephritis appears to be much less impaired than that of excreting nitrogen. Consequently a restriction in the chloride intake may fairly quickly restore the chlorides to normal.

Phosphates. According to the observations of Bloor (1918) the phosphoric acid compounds of human blood may be divided into two classes: *1*, the acid-soluble—soluble in dilute acids and precipitated with the proteins by alcohol-ether—and *2*, the lipoid-phosphoric acid compounds—soluble in alcohol-ether and precipitated with the proteins by dilute acids. These two groups are apparently sharply defined and since their sum is practically equal to the total phosphates, the presence of other forms of phosphorus in blood in significant amounts is doubtful.

Inorganic phosphates and an unknown compound which on decomposition by heating with acid yields phosphoric acid (hydrolyzable phosphoric) are present in the first group, while substances of the type of lecithin are found in the second group. Bloor gives the average content of inorganic phosphorus in the plasma of both men and women as about 3.0 mgm. per 100 cc. and of lipoid phosphorus about 7.5 mgm. According to Buell (1923), inorganic phosphorus is absent from the corpuscles. With this exception the corpuscles are relatively richer in all types of compounds than the plasma. The amount of the unknown form of phosphorus combination in the plasma is very small, but in the corpuscles it constitutes 60 to 80 per cent of the total phosphorus. This large amount of organic phosphorus in the corpuscles is significant considering the fact that Bloor has shown that "lecithin" formation takes place in the corpuscles during fat absorption. Furthermore it would appear to be the mother substance of the phosphoric acid of the lipoid phosphorus compounds. Owing to the fact that this organic phosphorus compound is relatively unstable, it is probably easily made available to serve as a buffer in case of need.

The presence of phosphorus in the blood in lipoid form has long been recognized, but exact data regarding the inorganic phosphorus are of more recent origin. Clinical interest is attached at present to the inorganic phosphorus. In 1915 Greenwald reported observations on the acid-soluble (largely inorganic) and lipoid phosphorus of human blood serum. He observed that normally the acid-soluble phosphorus as P varied between 2 and 6 mgm. per 100 cc., but that in severe nephritis it might be considerably increased. A year later Marriott and Howland (1916) confirmed these observations and pointed out that the retention of (acid) phosphate would seem to be sufficient to account for the degree of acidosis observed. Denis and Minot (1920) have studied the inorganic phosphorus of the plasma in a large series of pathological conditions. In conditions other than nephritis and cardio-renal disease figures varying from 1.2 to 3.1 mgm. of P per 100 cc. of plasma were found, while in one case of uremia figures exceeding 40 mgm. were observed. Feigl (1920) has likewise given figures for the acid soluble phosphorus (7.2 to 14.5 mgm.) in a series of nephritics. The introduction of simple colorimetric methods of phosphorus estimation at the hands of Bell and Doisy (1920) and others has greatly stimulated investigation in this field. de Wesselow (1923) has reported observations on 53 consecutive cases of nephritis with 13 deaths. In 12 of the deaths the highest P ranged from 10 to 22 mgm. He believes that phosphate retention is

more definitely connected with the symptoms of true uremia than is the retention of urea, and that the phosphorus is of great prognostic value. Schmitz, Rohdenburg and Myers (1924) have collected observations on a comparatively large series of nephritics, the highest figure for inorganic phosphorus being 25.5 mgm. In several cases low calcium figures, 4 to 5 mgm., were encountered, the phosphorus values being about 15 mgm. Where estimated, the serum proteins were low. These cases all showed uremic convulsions.

The observations which have been made regarding the inorganic phosphorus in rickets by Howland and Kramer (1921) and others are especially interesting and significant. Howland (1923) has recently summed up the findings: "The breast fed infant has an average of about 6 mgm. inorganic P per 100 cc. of serum, the average for the artificially fed is a little lower, about 5.5 mgm., while the rachitic infant has 3.5 mgm. or less. In one instance it was as low as 0.8 mgm. To this rule of the reduction of inorganic phosphorus, no exception has been seen. In the last 72 cases observed the average for the inorganic phosphorus has been 2.5 mgm., a reduction of more than 50 per cent. In the same cases the average for the calcium has been 9.6 mgm., a reduction of approximately 5 per cent. Whether the diet is woman's milk or cow's milk the result is the same." Howland points out that in rickets the bone is of normal composition, but that where the product of Ca × P is not 40 or above deposition of calcium phosphate does not occur. Cod liver oil and ultra violet light, the two therapeutic agents most widely used in the treatment of rickets, both raise the blood phosphorus. Zucker and Matzner (1924) have recently pointed out that the feces of rachitic rats are more alkaline than normal but that the two therapeutic agents above both lower the pH of the feces, thus promoting absorption of calcium and phosphorus.

Tisdall and Harris (1922) and Moorhead, Schmitz, Cutter and Myers (1923) have observed that following major fractures in adults there is a rise in the inorganic phosphorus content of the blood, in many instances to the level found in children. There appears to be a similar slight change in the calcium. The rise may take from several days to two to four weeks. After union the phosphorus gradually falls. As a rule cases with non-union do not show this reaction in the blood phosphorus following fracture.

Sulfates. The only figures so far reported for the sulfates of human blood are those of Denis (1921) and Denis and Hobson (1923). From their data it seems probable that the normal sulfate figures for serum

(or plasma) are in the neighborhood of 0.5 to 0.9 mgm. of S per 100 cc. of blood. In nephritis figures as high as 16 mgm. have been observed. Denis has pointed out that the sulfate ion is apparently excreted with difficulty so that in kidney insufficiency the concentration of this fraction may increase enormously, in some cases to 3,000 per cent above the normal value; in this respect the sulfate ion may be likened to the uric acid fraction although the percentage increases observed are far above any concentration of uric acid so far reported.

Blood gases. The practical importance of a knowledge of the factors concerned in the carrying of oxygen to the tissues and the removal of carbon dioxide is apparent. The most common use of the carbon dioxide estimation, however, is for quite a different purpose, namely, as a measure of the blood bicarbonate. Its use in this connection is discussed under Acid-Base Balance. The introduction by Van Slyke of accurate and relatively simple methods of blood gas analysis (see Van Slyke and Stadie, 1921) has made possible the collection of reliable data on the oxygen and carbon dioxide content of the blood in normal and in pulmonary and circulatory conditions.

Oxygen. The ability of the blood to absorb and take up oxygen depends upon its hemoglobin content. Since hemoglobin so readily takes up and gives off oxygen, it is obvious that venous blood should be partly unsaturated and therefore differ from the arterial blood in respect to its oxygen content, and further that blood obtained from different parts of the venous system should differ in its oxygen unsaturation. In the human adult the superficial veins of the limbs and neck, particularly the arms (vena medina), are the only sources from which venous blood can be obtained. This means that in the human only blood coming from a limited region, consisting chiefly of muscle, can be studied.

Lundsgaard (1918) found that in twelve normal resting adults the oxygen content of the venous blood ranged from 9.6 to 18.0 volumes per cent with an average of 13.6, while the oxygen unsaturation ranged from 2.7 to 9.0 volumes per cent with an average of 5.8. In studying this question on circulatory disorders Lundsgaard (1918a) found in twelve patients with compensated heart lesions that the unsaturation fell within normal limits, between 2.5 and 8 volumes per cent, while in four patients with uncompensated heart disease the values for the unsaturation were all above the normal limits, from 9.7 to 15.2 volumes per cent In such cases the oxygen unsaturation appears to afford an objective criterion of the positive effect of digitalis therapy. From studies per-

formed on patients with varying amounts of hemoglobin it has been shown that the oxygen unsaturation of the venous blood is independent of the oxygen capacity, unless the latter is reduced below the normal value for oxygen unsaturation (about 5 volumes per cent). Lundsgaard found, for example, that in a polycythemic patient with an oxygen capacity of 33.4 volumes per cent, the venous oxygen unsaturation was 5.4 volumes per cent, while in an anemic patient with an oxygen capacity of 6.7 volumes per cent the venous oxygen unsaturation was 5.2 volumes per cent, indicating that the tissues extract from the blood all the oxygen they need with apparently equal readiness, regardless of whether the extraction leaves a great oxygen reserve in the blood as in polycythemia, or practically no reserve as in anemia.

As pointed out by Lundsgaard (1919), the primary cause of cyanosis is an increase in the reduced hemoglobin or oxygen unsaturation of the blood in the peripheral capillaries. When the mean capillary oxygen unsaturation, which is calculated as the mean between venous and arterial unsaturation, and is normally about 2 to 3 volumes per cent is increased to 6 to 7 volumes per cent, cyanosis appears. The increased mean capillary oxygen unsaturation may be produced in two ways, either by an abnormally great reduction during passage through the capillaries or by a state or partial reduction in the arterial blood entering the capillaries. The first condition occurs during exercise, or when the blood flow is retarded, as in decompensated heart conditions. The second condition (partial arterial unsaturation) occurs in certain lung and heart diseases, and when the alveolar oxygen tension is greatly decreased, as at high altitudes. Cyanosis cannot be produced in patients whose hemoglobin is below about 5 per cent.

Considerable additional information may be obtained when the study of the oxygen content of the arterial blood is included. Such studies have been conducted on normal and certain respiratory and circulatory conditions by Stadie (1919), by Harrop (1919) and by Stewart (1921), the arterial blood being obtained from the radial artery. Observations obtained by Stadie for the arterial and venous oxygen, and total oxygen capacity of five normal resting men averaged as follows: oxygen capacity, per 100 cc. of blood, 21.2 cc., arterial oxygen content, 20.2 cc., and venous oxygen content, 15.6 cc. This gives an arterial unsaturation of 1.0 cc. or 5.0 per cent and a venous unsaturation of 5.6 cc. or 26.8 per cent. Similar studies were made on a series of pneumonia cases (chiefly post-influenza), a high arterial unsaturation being observed in the fatal cases. A definite relation was found to exist between the degree of

cyanosis and the per cent of arterial unsaturation. Record of the clinical cyanosis, in correlation with the blood findings, was made by means of colored illustrations. With increasing cyanosis the arterial unsaturation becomes greater. The venous saturation varies similarly. Obviously the cyanosis of pneumonia patients is due to the incomplete satura-

TABLE 5

The oxygen unsaturation of arterial and venous blood in pneumonia (Stadie)

TYPE OF CASES	NUMBER OF CASES	ARTERIAL UNSATURATION			VENOUS UNSATURATION		
		Maximum	Minimum	Mean	Maximum	Minimum	Mean
Normal individuals	5	6.5	2.8	5.0	33.0	22.7	26.8
Non-fatal cases	16	33.0	1.6	13.9	61.2	14.4	36.3
Fatal cases	16	68.2	14.1	32.0	85.5	22.3	57.0

TABLE 6

Oxygen capacity and arterial and venous content of oxygen and carbon dioxide (Figures of Harrop)

INDIVIDUAL	OXYGEN CAPACITY	OXYGEN CONTENT OF ARTERIAL BLOOD	PERCENTAGE ARTERIAL SATURATION	ARTERIAL OXYGEN UNSATURATION	OXYGEN CONTENT OF VENOUS BLOOD	OXYGEN CONSUMPTION	CO_2 CONTENT OF ARTERIAL BLOOD	CO_2 CONTENT OF VENOUS BLOOD
	vol. per cent	*vol. per cent*		*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*
1	23.7	23.0	98	0.7	17.6	6.4	51.8	57.2
2	17.2	17.2	100	0.0	14.6	2.6	54.7	56.7
3	16.3	15.3	94	1.0	10.5	4.8	52.9	55.9
4	20.6	19.8	96	0.8	13.5	6.3	46.5	51.7
5	18.7	17.8	95	0.9	15.1	2.7	44.8	48.3
6	20.6	19.8	96	0.8	12.7	7.1	49.7	54.6
Average	19.5	18.8	96	0.7	14.0	5.0	50.1	54.1
Normal adult, resting	22.0	21.1	96	0.9	15.1	6.0	53.3	56.9
After exercise	22.4	19.2	86	3.2	12.9	6.3	32.3	41.1

tion of venous blood with oxygen in the lungs. The range of arterial and venous unsaturation encountered in fatal and non-fatal cases of pneumonia is well shown in table 5, compiled from Stadie. As will be noted, the unsaturation of the fatal cases averaged 32 per cent and in one case reached 68 per cent, the venous unsaturation exceeding 85 per cent.

The oxygen content of the arterial blood in anemia and heart disease had been studied by Harrop, who likewise made a careful study of both the blood gases (oxygen and carbon dioxide) in the arterial and venous blood of fifteen normal subjects, his figures for oxygen agreeing closely with those of Stadie. Figures illustrating his findings in six normal individuals and one individual before and after exercise are given in table 6. Low values were found for the oxygen content of the venous blood, but the normal oxygen consumption was maintained. No deviations from the normal were found in the arterial and venous blood from cardiac patients without arrhythmias, well compensated, and at rest in bed. With cardiac cases showing varying degrees of decompensation, particularly when there are physical signs of pulmonary congestion, there is a disturbance of pulmonary exchange, as indicated by a lowering of the percentage saturation of the arterial blood with oxygen.

Stewart has studied the blood gases in auricular fibrillation and after the restoration of the normal mechanism under the action of quinidine sulfate. The cases showed a definite increase in venous oxygen saturation and a diminution in the coefficient of utilization and in oxygen consumption with the onset of normal mechanism, which was interpreted as due to an improvement in blood flow. In several cases with a high oxygen capacity there was a definite fall in oxygen capacity after the onset of the normal mechanism, indicating that the high hemoglobin was a compensatory mechanism.

Carbon dioxide. It has long been recognized that the blood not only carries oxygen to the tissues, but also returns carbon dioxide to the lungs. How is this carbon dioxide carried? Recent studies of L. J. Henderson (1920), Haggard and Y. Henderson (1920), Smith, Means and Woodwell (1921) and Van Slyke (1921) indicate that the hemoglobin plays quite as important a rôle in the transport of CO_2 as O_2 The carbon dioxide carriers of the blood was the topic of Van Slyke's review in 1921. According to Van Slyke 80 to 95 per cent, sometimes possibly all, of the alkali furnished to neutralize the CO_2 that enters the venous blood, comes from hemoglobin. Doisy, Briggs, Eaton and Chambers (1922) have obtained similar figures. Of such alkali, the greater part is set free when the relatively strong acid, oxyhemoglobin, is changed by loss of oxygen to the much weaker one, reduced hemoglobin. The hemoglobin from its location in the cell is able to increase the alkali content of the plasma by withdrawing Cl into the cell from the plasma NaCl, thereby leaving the Na to form $NaHCO_3$.

Arterial blood of man normally contains about 50 volumes per cent of carbon dioxide, and the venous blood 55 to 60 (Van Slyke). As will be noted in table 6 taken from Harrop, the CO_2 content of the arterial blood in the six resting individuals tabulated averages about 50 volumes per cent, while that of the venous blood is 4 volumes per cent higher. After 15 minutes of brisk exercise Harrop found the CO_2 content of both arterial and venous blood reduced, with a considerable increase in the venous-arterial whole blood difference. The oxygen consumption was, however, only slightly increased. Smith, Means and Woodwell (employed Haldane-Henderson apparatus) found the CO_2 content of eight normal whole bloods to average 50.4 volumes per cent, while the venous blood showed 58.7 volumes per cent, a difference of 8.3. As the blood, passes from the arterial to the venous side of the circulation in normal man its cells gain from 4 to 11 volumes per cent of CO_2 according to their analyses, while the corresponding gain in the plasma is only from 0 to 1.8 volumes per cent, indicating that the transport is accomplished mainly in the cells.

ACID-BASE BALANCE. Obviously one of the most fundamental factors determining the acid-base balance of the blood is the actual balance between the fixed acids and bases. Kramer and Tisdall (1922) have made a careful study of the distribution of the basic radicles between the cells and serum, and with the aid of recorded figures for the acid radicles (-Cl, -HCO_3, -HPO_4, -SO_4) have endeavored to ascertain the balance. They point out that the corpuscles of human blood do not contain appreciable amounts of sodium or calcium, but are rich in potassium, while the concentration of magnesium is slightly higher in whole blood than in serum. Of the fixed bases of the serum, sodium constitutes about 92 per cent and potassium practically all that of the corpuscles. There is an excess of about 16 per cent of basic radicles, over the well known acid radicles in both serum and corpuscles, probably in combination with protein.

Since the acid-base equilibrium has so recently been discussed and the literature reviewed by Van Slyke (1921), the Haemoglobin Committee (1923) and Wilson (1923), it will be considered here very briefly. Van Slyke and his co-workers, in particular, have done much to advance our knowledge of this problem. They have given us many new methods, which, in their hands and the hands of others, have supplied many useful data.

In health, the blood is uniformly maintained at a constant slightly alkaline reaction through the influence of the bicarbonate, phosphate and

proteins of the blood. The chloride shift, whereby the Cl ion may pass through the cell membrane leaving the Na ion in the plasma to serve as base, is likewise a factor of importance. The bicarbonate is, however, the most important factor in regulating the reaction of the blood since the normal blood bicarbonate is able to furnish approximately three times as much alkali as all other buffers combined.

In 1917 Van Slyke and Cullen described a simple method of measuring the so-called "alkaline reserve" of the body by estimating the plasma bicarbonate. This method quickly found extensive application in the study of pathological conditions. Van Slyke and Cullen recognized at the time that the plasma bicarbonate would serve as an adequate index of acidosis or alkalosis only so long as these conditions remained compensated, i.e., the cH remained normal, but it was believed then that the cH was a physiological constant and did not change appreciably until shortly before death. Subsequent studies by a number of different investigators showed, however, that uncompensated conditions do occur, not only in disease but also in normal individuals as a result of altered pulmonary ventilation or exercise. Van Slyke (1921) was the first to clearly correlate the factors involved in the normal and abnormal variations in the acid-base balance of the blood. He pointed out that there are nine theoretically possible variations depending on the fact that the blood bicarbonate might be high, low or normal, and in each of these conditions the pH might be high, low or normal. As thus classified only one condition can be considered as normal, that in which both blood bicarbonate and pH are within normal limits.

The CO_2 content of normal human blood plasma under 40 mm. C_2O tension at 38° appears to fall between 51 and 63 volumes per cent, and of this Van Slyke and Cullen (1917) have shown that 19/20 is in the form of bicarbonate. When the CO_2 tension is other than 40 mm. the normal bicarbonate figures also change. Quite obviously the pH is a function of the ratio between the $NaHCO_3$ and the H_2CO_3. In order to ascertain which one of the nine possible variations exist in the blood *in vivo*, it is simply necessary to determine two of the three involved variables, namely, pH, $NaHCO_3$ and H_2CO_3. With any two of them the third can be determined by a diagram such as employed by Van Slyke (1921). The acid-base balance may be most easily ascertained by estimating the pH and CO_2 content of the same sample of blood plasma. For the CO_2 determination the gasometric method of Van Slyke (1917) is available, while Cullen (1922) has described an accurate colorimetric method of measuring the pH. Myers, Schmitz and Booher

(1923) have simplified the technique of the latter method and increased its delicacy. Stadie and Van Slyke (1920) have shown that the venous

TABLE 7

Some abnormal variations in the acid-base balance (Myers and Booher)

AREA	CASE	DATE 1923–24	pH	CO_2 CONTENT	REMARKS
				vol. per cent	
I	1	4/9	7.52	83	Cholecystectomy, receiving $NaHCO_3$
	2	10/29	7.53	70	Duodenal ulcer—Sippy treatment
		10/31	7.53	70	Duodenal ulcer—Sippy treatment
		11/2	7.52	70	Duodenal ulcer—alkali discontinued 10/31.
	3	11/21	7.57	62	Carcinoma of stomach—X-ray therapy
	4	12/5	7.60	75	Diabetes, receiving $NaHCO_3$
	5	12/17	7.52	70	Persistent vomiting
	6	12/17	7.53	63	Nephritis, receiving alkali
	7	1/24	7.54	88	Intestinal obstruction—persistent vomiting
II and III	8	11/26	7.51	61	Diabetic with gangrene—Temp. 102°F.
	9	5/10	7.47	50	Temp. 100°F.
IV	10	7/3	7.40	75	Gastric ulcer, receiving alkali
	11	11/22	7.38	72	Duodenal ulcer, receiving alkali
V			7.35–7.43	55–65	Normal variations
VI	12	5/22	7.37	37	Diabetes
	13	9/10	7.37	33	Nephritis
VII and VIII	14	4/20	7.30	63	Cardiac decompensation
	15	6/13	7.33	62	Chronic tuberculosis—auricular fibrillation
IX	16	6/5	7.13	10	Diabetic coma
		6/6	7.28	38	After administration of insulin
	17	6/4	7.17	13	Carcinoma of kidney
		6/8	6.98	4	Carcinoma of kidney
	18	5/8	7.15	25	Chronic nephritis—urea N. 88 mgm.
		5/28	7.16	19	Chronic nephritis
		6/1	7.06	17	Chronic nephritis

blood plasma may be used directly for the CO_2 determination (in measuring the alkaline reserve) without resaturation with carbon dioxide,

if the blood is centrifuged and the plasma analyzed without escape of CO_2.

Myers and Booher (1924) have recently determined the acid-base balance of the blood in more than 200 hospital cases and have encountered conditions falling into each of the nine areas described by Van Slyke. Their data indicate that an abnormal acid-base balance occurs more frequently than has hitherto been supposed and that the recognition of these conditions is of great clinical importance. The estimation of the CO_2 capacity of blood plasma was originally recommended by Van Slyke and Cullen (1917) as a measure of the alkali deficit occurring in compensated acidosis. For this purpose it appears to be quite adequate so long as the CO_2 remains above 35 volumes per cent, while below this level it may be assumed that the condition is uncompensated. The use of the test in this connection has been of very great clinical value. For other variations in the acid-base balance it is generally inadequate.

Table 7 taken from observations of Myers and Booher, gives an idea of some of the abnormal variations in the acid-base balance which may be encountered clinically. The data are arranged according to Van Slyke's nine areas and will be discussed on that basis.

Area 1, characterized by a high pH and a high plasma bicarbonate, has been encountered particularly after the administration of $NaHCO_3$ in large quantities and as a result of loss of HCl through persistent vomiting. Obviously the ratio of $NaHCO_3$:H_2CO_3 is altered by an increase of $NaHCO_3$. Binger, Hastings and Neill (1923) have reported alkalosis in a case of pneumonia receiving alkali, the pH being 7.55. It may also be noted that Hussey (1922) has observed a condition of uncompensated alkali excess in rabbits following x-ray exposure. Cases with acid-base findings falling in area 1 are shown in table 7, the alkalosis being due to bicarbonate therapy. The highest pH recorded is 7.60, occurring in a case of diabetes following $NaHCO_3$ administration. The CO_2 content of this plasma was 75. It is of interest to note that acetone continued to be excreted by the kidneys at this time. The influence of radium on the acid-base balance would appear to be analogous to the action of x-rays. Hardt and Rivers (1923) have reported bad results following Sippy treatment in certain cases of duodenal ulcer. The cases showed impaired renal function and apparently suffered from alkalosis, although no pH figures were given. A case of duodenal ulcer which developed alkalosis on Sippy treatment is shown in table 7.

Alkalosis is a condition, which in the past has frequently been overlooked. Care should always be exercised in the administration of

alkali. In cases with impairment of renal function it is a dangerous procedure unless the acid-base balance is carefully followed. Means, Bock and Woodwell in 1921 cautioned against the administration of large amounts of bicarbonate to such cases. That the CO_2 content is not always an adequate guide, is evident from the fact that after the administration of $NaHCO_3$ has been discontinued the pH sometimes returns to normal in advance of the CO_2 while in other instances the reverse is true.

Areas 2 and 3 are characterized by an abnormally high pH and a normal or low CO_2 content. Cases of overventilation as a result of fever are included in these areas, the rapid loss of H_2CO_3 being the responsible factor in changing the $NaHCO_3$:H_2CO_3 ratio from its normal balance. In fevers the increase in elimination of H_2CO_3 need be only relatively small to affect this ratio. Koehler (1923) reported this condition in cases of fever and pointed out its similarity to over-ventilation due to the immersion of individuals in warm water. (Findings for two illustrative cases are shown in table 7.) Voluntary over-ventilation for a short period of time is said to cause a greater degree of alkalosis than has ever been reported as a condition of pathological occurrence.

Area 4 is characterized by a normal pH and a high plasma bicarbonate. This condition has been encountered after the administration of $NaHCO_3$, for example, in cases undergoing Sippy treatment. In case 10, table 7, a plasma CO_2 content of 75 is associated with a normal pH of 7.40. The buffering action of the blood and the extent to which H_2CO_3 can be retained to balance the increase of $NaHCO_3$ probably determines its capacity to remain compensated. In emphysema Scott (1920) has apparently observed a state of compensated CO_2 excess. In one case he reported a CO_2 content of 82.7 cc. with a normal pH of 7.4.

Area 5 comprises the normal range of acid-base balance of the blood. The recent studies of Myers and Booher (1924) indicate that the pH of normal individuals at rest and at sea level falls between 7.35 and 7.43 and that pH values below 7.32 and above 7.47 are definitely abnormal. In this area the values for the bicarbonate content of plasma range from 55 to 65 volumes per cent.

Area 6 represents conditions of compensated alkali or CO_2 deficit, the pH being normal and the plasma bicarbonate low. This area includes those cases termed compensated acidosis. This condition has been encountered in cases of diabetes in which the abnormal acid production is not sufficiently rapid to overtax the compensating powers of the organism and in cases of renal impairment in which the elimination of acid

radicles is not sufficiently decreased to cause a change in blood reaction. A representative case of each group is given in table 7. The excess production of abnormal acids or the decreased elimination of normal acid radicles results in the displacement of the $\overline{HCO_3}$ of the blood bicarbonate with a consequent decrease in the capacity of the blood as a carrier of CO_2. Hence a more rapid ventilation rate is necessary to rid the body of the CO_2 constantly being formed.

Areas 7 and 8 include conditions of uncompensated CO_2 excess, characterized by a low pH and a high or normal CO_2. Peters and Barr (1921) reported observations of this condition in cases of cardiac decompensation, with dyspnea in which there was evidence that the efficiency of the lungs as a means for the oxygenation of the blood and the elimination of CO_2 was greatly reduced. The minimum pH value of venous blood recorded by these authors was 7.17. Two cases typifying this area are given in table 7; one being a case of cardiac decompensation and the other a case of chronic tuberculosis with auricular fibrillation. The change in $NaHCO_3$:HCO_3 ratio results from an increase of H_2CO_3 in the blood due to its decreased elimination by the lungs.

Area 9 represents a state of uncompensated acidosis and is characterized by a low pH and a low plasma bicarbonate. This condition may be the result either of the formation of abnormal acids beyond the power of the organism to compensate or of the impairment of the function of the kidney to eliminate normal acids or of the function of the kidney to form ammonia. The first group is encountered in cases of diabetes in which β-hydroxybutyric and aceto-acetic acids are formed in excess of the ability of the body to compensate by buffer action or in excess of the ability of the kidney to eliminate them with sufficient rapidity as acids or neutralized salts. Cullen and Jonas (1923) have recently reported observations on the influence of insulin on the acid-base balance of diabetic acidosis. The plasma pH and bicarbonate were found to return to normal coincidently.

The acidosis resulting from impaired renal function is due to a retention of acid radicles which are eliminated by the normal kidney. Two cases representing this condition are reported in table 7. It is interesting to note that the case of chronic nephritis lived for many days in a condition of uncompensated acidosis.

Clinical deductions. The chemical studies carried out on the blood during the past ten years have advanced scientific medicine in several different ways, and may be grouped as follows: *1*, they have given us a clearer insight into many physiological functions; *2*, they have

supported certain clinical observations with scientific facts, and *3*, they have furnished new methods of recognizing and measuring the severity of disease.

Recognition, for example, of the direct absorption of amino acids by the blood after protein digestion, of the relation between the blood sugar level and glycosuria, and of the factors enabling hemoglobin to transport O_2 and CO_2 fall in the first group, while similarly the correlation of the degree of oxygen unsaturation of the blood with clinical observations on cyanosis belongs to the second group. In the past the practical usefulness of biochemistry has been confined chiefly to these two groups, but the many new methods of blood analysis also furnish information of value in the diagnosis, prognosis and treatment of disease.

Some of the more important clinical uses of chemical blood analysis may be summarized. Excepting the time honored hemoglobin estimation practically all of the determinations are new.

Since only moderate impairment of renal function has a definite influence on the level of the blood urea, it constitutes the most valuable measure of renal function at our disposal. The creatinine of the blood is of greater prognostic value than the urea in cases of advanced nephritis, since it is only retained when renal function is greatly impaired. Uric acid is more difficultly eliminated than either urea or creatinine. For this reason a rise in the blood uric acid occurs earlier than in the case of urea, although uric acid retention is a less reliable sign of impaired renal function. It may, however, furnish very reliable information in the diagnosis of gout.

The degree of hyperglycemia in diabetes not only serves as an index of the severity of the condition, but also as a guide to the treatment. Glycosuria is often a very uncertain index of the hyperglycemia, owing to the rise in the sugar threshold which generally occurs in advanced diabetes.

Cholesterol serves as an index of the degree of lipemia in diabetes. In nephrosis the hypercholesterolemia is almost diagnostic.

Infantile tetany is dependent upon a definite reduction in the calcium content of the blood serum, while in rickets there is a reduction in the content of inorganic phosphorus. A marked retention of inorganic phosphorus occurs in the terminal stages of chronic nephritis, the amount of the retention being of considerable prognostic value.

As a measure of the acidosis due to alkali deficit, such as occurs in diabetes, nephritis and the marasmus of infants, the CO_2 content of

the blood is invaluable. For other disturbances of the acid-base balance the CO_2 alone is generally inadequate, figures for the pH generally being necessary to secure entirely reliable information.

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